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**CLINICAL AND PROPHYLACTIC STUDIES OF HUMAN
TUBERCULOSIS IN A LOW-ENDEMIC SETTING**

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Clinical and prophylactic studies of human tuberculosis in a low-endemic setting

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Sweden is a low burden country for tuberculosis (TB). New cases occur mainly among immigrants from countries with a higher TB prevalence. Most persons infected with TB (latent TB) will not develop disease (active TB). Prolonged treatment is necessary and can cause severe adverse drug reactions. A well-functioning TB program is essential to interrupt the transmission of infection in the community. The increasing global problem of resistant TB-strains necessitates development of a new tuberculosis vaccine that is more effective than the Bacillus Calmette Guerin (BCG) vaccination that has long been in use.

Globally, human immunodeficiency virus (HIV) is the single strongest medical risk factor for active TB. The implementation of anti-retroviral treatment (ART) in 1996 completely changed the prognosis for persons living with HIV. By restituting the immune defense, ART has provided a strong protective effect against active TB. ART in combination with anti-TB treatment entails a higher risk of adverse drug reactions and this risk is even greater if ART is introduced during TB treatment. Sweden is a low burden country for HIV and more than 90% of all HIV-infected individuals in Sweden receive effective ART.

In **paper I** we described the socio-demographic and clinical characteristics of the 127 HIV-infected persons that developed active TB in Stockholm County 1987–2013. The majority of the patients in the co-infected cohort were foreign-born (87%). After the introduction of ART in 1996 the success of TB treatment increased from 65% to 91%. In patients diagnosed with co-infection after 1996, treatment success was predicted by ART treatment (odds ratio (OR) 13.3, 95% confidence interval (CI) 1.5–114.8) and a CD4⁺ cell count at TB diagnosis >200 cells/ μ l (OR 17.2, 95% CI 1.2–236.6). Adverse reactions severe enough to lead to modification of anti-TB treatment occurred in 23% of the patients diagnosed with co-infection after 1996, and the risk of adverse events was significantly increased if ART was introduced after TB diagnosis (OR 13.3, 95% CI 1.6–112.4).

BCG, the TB vaccine used since the 1920s, is most effective against active disease in children but does not give adequate protection in adults. In **paper II** we performed a phase 1 study, investigating the safety and the immunogenicity of the new vaccine candidate H4:IC31, consisting of a fusion protein of two TB antigens (Ag85B and TB10.4) and an adjuvant (IC31). In two randomized and double-blinded studies, conducted in Sweden and Finland, including BCG-vaccinated healthy individuals, 125 study subjects were immunized twice with different doses of antigen and adjuvant or placebo. The vaccine was well tolerated with only mild to moderate, mainly self-limiting adverse events: injection-site pain, myalgia, arthralgia, fever and post-vaccination inflammatory reaction at the site of screening tuberculin skin test injection. The vaccine triggered an antigen-specific and multifunctional CD4⁺ cell response and cytokine production, most prominent after two doses of 5, 15 or 50 μ g of H4 combined with 500 μ g of IC31.

Latent TB infection (LTBI) is defined as a detectable immune response against TB without signs or symptoms of active disease. Treatment for LTBI is recommended by the Public Health Agency of Sweden to prevent active TB in persons with untreated HIV-infection. In contrast, the National Reference Group for Antiretroviral therapy in Sweden (RAV) recommends neither screening nor treatment for LTBI in this group, with reference to Sweden's well-functioning HIV care; almost all HIV-infected persons are offered ART and if they nevertheless develop active TB the close follow-up of this group is considered sufficient for early detection and initiation of TB treatment. In **paper III** we studied the incidence of and risk factors for active TB in persons living with HIV in Stockholm County, 1996–2016. We observed an overall incidence rate of active TB of 6.2 cases (95% CI 5.1–7.6) per 1 000 person-years with a significant decline over the study period. Originating from a TB-endemic region

was the only characteristic associated with a higher risk of active TB (Hazard Ratio (HR) 8.84 (95% CI 3.09–23.61)). The number of patients needed to treat for LTBI to prevent one case of active TB among patients from TB-endemic regions was 22 (95% CI 26–47). Although the incidence of TB declined significantly during the study period, it was still 80 times higher than in the general population at the end of the study.

Recurrence of infection after completed antibiotic treatment is reported to occur in around 2% of TB patients, in low-endemic settings. Recurrence can be caused by relapse of infection or reinfection by another TB strain. Molecular typing with whole genome sequencing (WGS) can distinguish relapse from reinfection with a high resolution. As an evaluation of current treatment strategies and treatment control, **study IV** was aimed to analyze the frequency of TB recurrence in Stockholm County, 1996–2016. Recurrence was defined as a new TB infection more than 180 days after successful treatment completion. The recurrence frequency was 0.7% in 2,552 patients diagnosed with culture-verified TB. With WGS analysis, 71% were classified as relapse cases. Drug-resistant TB was present in 50% of the patients with relapse. No acquired drug resistance was detected with WGS comparing the isolates in relapse cases.

In conclusion, several interventions are needed to further reduce the incidence rate of TB in Sweden. The introduction of ART in 1996 has dramatically enhanced the success rate of TB treatment in patients co-infected with HIV and TB (**Paper I**). Since the introduction of ART, the incidence of active TB in persons living with HIV in Stockholm County has also declined significantly. However, our data indicate that the addition of screening and treatment of LTBI in persons with HIV could be expected to further decrease the incidence of TB in persons from TB-endemic regions (**Paper III**). Stockholm has a low TB relapse frequency, indicating a well-functioning TB care. Relapse occurs mainly among patients with resistant TB, which should be considered in the follow-up of these patients (**Paper IV**). The new vaccine candidate H4:IC31 is safe and immunogenic. Encouraging results from a phase 2 study of the vaccine candidate performed in South Africa were presented in 2018 (**Paper II**).

LIST OF SCIENTIFIC PAPERS

- I. Wannheden C, Norrby M, Berggren I, Westling K. Tuberculosis among HIV-infected patients in Stockholm, Sweden, 1987-2010: treatment outcomes and adverse reactions *Scand J Infect Dis*. 2014 May;46(5):331-9. doi: 10.3109/00365548.2013.878033. Epub 2014 Feb 11. PMID: 24512373
- II. Norrby M, Vesikari T, Lindqvist L, Maeurer M, Ahmed R, Mahdavi S, Bennett S, McClain JB, Shepherd BM, Li D, Hokey DA, Kromann I, Hoff ST, Andersen P, de Visser AW, Joosten SA, Ottenhoff THM, Andersson J, Brighenti S. Safety and immunogenicity of the novel H4:IC31 tuberculosis vaccine candidate in BCG-vaccinated adults: two phase I dose escalation trials *Vaccine*. 2017 Mar 14;35(12):1652-1661. doi: 10.1016/j.vaccine.2017.01.055. Epub 2017 Feb 17. PMID: 28216183
- III. Norrby M, Wannheden C, Ekström AM, Berggren I, Lindquist L. Incidence of tuberculosis in persons living with HIV in Stockholm during the era of anti-retroviral therapy 1996-2013. *Infect Dis (Lond)*. 2018 Oct 26:1-10. doi: 10.1080/23744235.2018.1486511. [Epub ahead of print] PMID: 30362392
- IV. Norrby M, Goenheit R, Mansjö M, Zedenius I, Vesterbacka J, Lindquist L, Berggren I. Whole genome sequencing of recurrent tuberculosis in Stockholm County 1996-2016. In manuscript.

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LIST OF ABBREVIATIONS

AE	Adverse event
Ag85B	Antigen 85B
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ART	Antiretroviral treatment
AUC	Area under the curve
BCG	Bacillus Calmette-Guerin
CDC	Center for Disease Control and prevention
CFP-10	Culture filtrate protein 10
CT	Computer tomography
CTLs	Cytolytic CD8+ T cells
DOT	Directly observed treatment
DTH	Delayed-type hypersensitivity
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
ESAT-6	Early secretory antigen target 6
FASCIA	Flow-cytometric Assay for Specific Cell-mediated Immune-response in Activated whole blood assay
^{18}F -FDG	^{18}F -fluorodeoxyglucose
FI	Fusion inhibitor
ICS	Intracellular cytokine staining
IL	Interleukin
IFN- γ	Interferon- γ
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
INI	Integras inhibitor
INR	International normalized ratio
IP-10	Interferon gamma-induced protein 10
IRIS	Immune reconstitution inflammatory syndrome
IQR	Interquartile range
HIV	Human immunodeficiency virus
LAM	Lipoarabinomannan
LTBI	Latent tuberculosis infection
MDR-TB	Multidrug-resistant tuberculosis

MIRU-VNTR	Mycobacterial interspersed repetitive unit-variable number of tandem repeat
MHC	Major histocompatibility complex
<i>M.tb</i>	<i>Mycobacterium tuberculosis</i>
NK-cells	Natural killer cells
NNS	Number needed to screen
NNT	Number needed to treat
NNRTI	Non nucleoside reverse-transcriptase inhibitors
NRTI	Nucleoside reverse-transcriptase inhibitors
NTM	Non-tuberculosis mycobacteria
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PET	Positron emission tomography
PI	Protease inhibitor
PPD	Purified Protein Derivative
PPV	Positive predictive value
PY	Person-years
QFT	QuantiFERON-TB Gold Plus
RAV	The Swedish reference group for antiretroviral therapy
RFLP	Restriction Fragment Length Polymorphism
RIF	Rifampicin
SNP	Single nucleotide polymorphism
TST	Tuberculin skin test
TB	Tuberculosis
Th1-cells	T-helper 1 cells
TNF- α	Tumor necrosis factor- α
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis

1 BACKGROUND

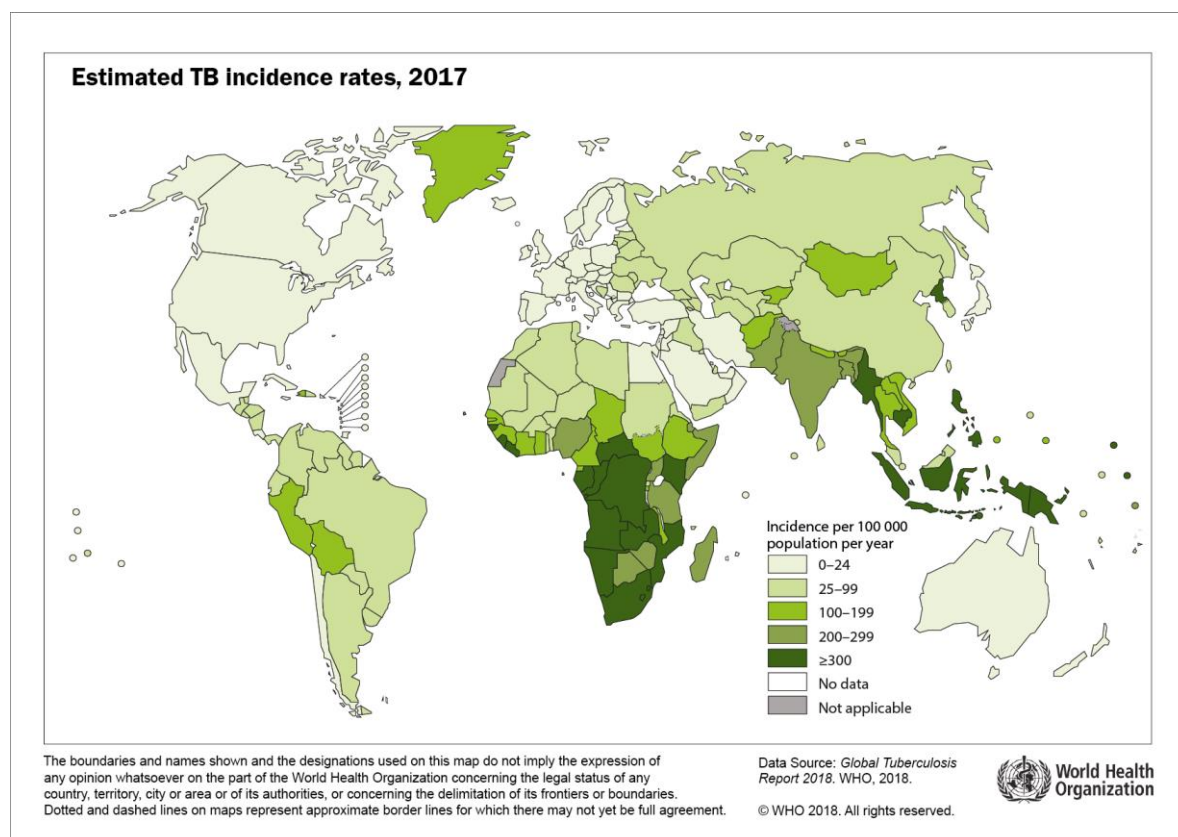
In 2014 the World Health Organization (WHO) set a goal to reduce the global tuberculosis (TB) incidence with 90%, by 2035 (1). The major means to achieve this goal include preventive treatment of people with high risk of disease, early TB diagnosis and treatment of all people with TB, and collaborative care for people with human immunodeficiency virus (HIV) and TB (1). In TB low-endemic settings (<100/100.000 inhabitants) such as Sweden, a majority of TB cases result from progression of latent TB infection (LTBI) rather than from local transmission (2). Efforts should be concentrated on the prevention of disease in vulnerable groups with high risk of progression from LTBI to active disease: migrants from TB-endemic settings; people with recent infection (especially children aged <5 years); and people with impaired immunity (e.g. owing to HIV infection or immunosuppressive treatments); but also through Bacillus Calmette-Guerin (BCG)-vaccination of infants at risk for TB (2).

TB is a contagious disease, caused by the bacillus *Mycobacterium tuberculosis* (*M.tb*) (3). The disease has been a curse since the beginning of human history with a high mortality rate but also with social implications and stigma (4). Crowding, poverty and malnourishment have always been associated with TB. Socioeconomic development and welfare can reduce the incidence of disease (5). With the intention to prevent TB infection, the BCG-vaccine was developed by Calmette and Guerin in the 1920s (4). The degree of efficacy of the vaccine is not well characterized, but it provides a strong protection against miliary and meningeal TB in infants (6). The vaccine was widely used in Europe from the 1940s and it contributed to the reduction of the epidemic, but improved economy, welfare and the segregation of infectious cases in sanatoria probably explain most of the decline in TB incidence before the implementation of active medical treatment in 1950s (4, 7). In areas with access to proper treatment, the incidence of TB continued to fall until the 1990s, when the spread of HIV fueled the epidemic (8).

1.1 EPIDEMIOLOGY

In 2019, TB remains a major cause of morbidity and mortality globally. In 2017, 10 million people were diagnosed with TB and 1.6 million died from the disease worldwide, according to the WHO (9). **Figure 1.** A major driver of the disease is HIV-co-infection and multi-drug resistant TB (MDR-TB) (9, 10). TB is a global concern: although primarily affecting high-incidence settings in Asia and Africa, with globalization and migrating populations it also reaches low-endemic countries (9, 11, 12). During recent years (2013–2017) the incidence of TB has been falling by an average of 2% per year globally and in Europe and the African region by 4–5% per year (9).

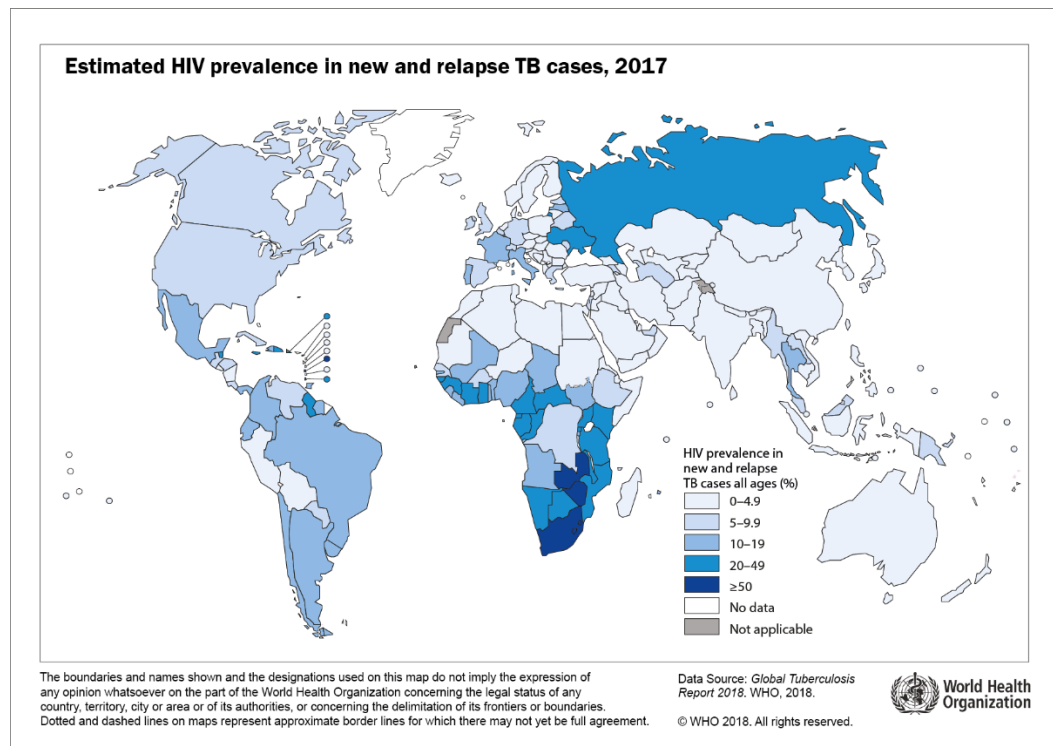
Figure 1. Global incidence of tuberculosis 2017



1.1.1 Tuberculosis and HIV

In 2017, 9% of all cases of active TB in the world occurred in persons living with HIV (a majority in sub-Saharan Africa) and TB was the major cause of mortality, with 300,000 deaths, in dually infected individuals (9). **Figure 2.** With increasing access to antiretroviral treatment (ART) against HIV in combination with better access to both HIV and TB diagnosis and treatment, the number of deaths among people living with HIV has fallen by 20% from 2015–2017 but still in 2017 the global access to ART was only 40% (9). The risk for TB activation and disease has been shown to be strongly reduced by early start of ART after HIV diagnosis (13).

Figure 2. Prevalence of HIV in tuberculosis cases 2017



1.1.2 Drug-resistant tuberculosis

Resistant TB is an increasing problem, with the highest burden in India, China and the Russian Federation (9). The degree of resistance ranges from mono-drug resistance to first line drugs (most commonly isoniazid (INH) and rifampicin (RIF) resistance), to MDR-TB (*M.tb* strains resistant to both INH and RIF), to extensively drug-resistant TB (XDR-TB) (MDR-TB plus resistance to fluoroquinolones and at least one injectable second-line drug) and they are all a major threat (14). In 2016 >18% of new cases in the Russian Federation were infected with a RIF-resistant or MDR-TB strain (15). Also in Sweden we observe a growing proportion of patients with MDR-TB, although still around 3% (16).

1.1.3 Tuberculosis in Sweden

In the beginning of the 20th century Sweden was a high-burden country for TB, with over 300 cases per 100,000 inhabitants. After the end of World War II the incidence fell dramatically with improved socio-economic welfare and fell even more after effective medical treatment became accessible (16). Sweden has been a low-burden country since the 1950s. **Figure 3.** With increasing immigration from TB-endemic regions (>100/100,000 inhabitants) the last twenty years, foreign-born patients represent >90% of all cases and 1996–2016 the median incidence was 6.0 cases per 100,000 population and year (16). **Figure 4.** Patients with TB are concentrated to the large cities with one third in Stockholm (16). TB is a notifiable disease in Sweden and it is mandatory to report TB-cases in the National Reporting System and Registry for Communicable Diseases (SmiNet), according to the Communicable Disease Act (16). The Public Health Agency of Sweden is responsible for surveying the disease and for storage of clinical strains.

Figure 3. Incidence of tuberculosis in Sweden 1940–2018. (The Public Health Agency of Sweden)

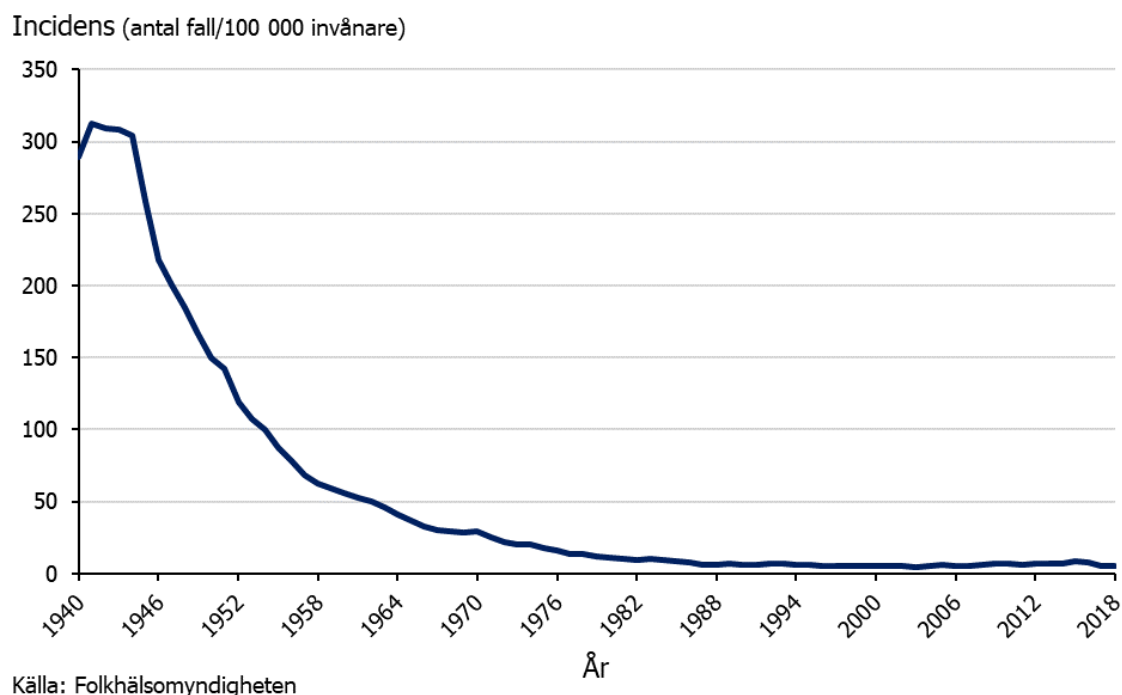
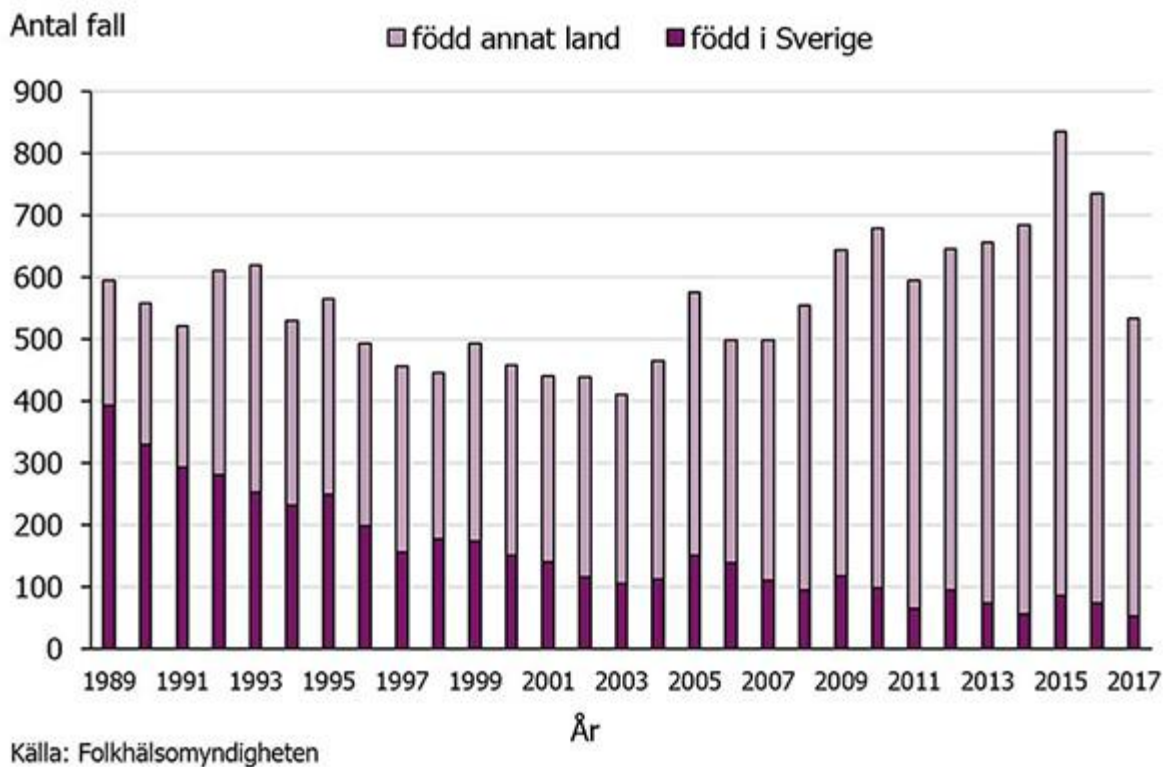


Figure 4. Number of tuberculosis cases in Sweden 1989–2017. Persons born in Sweden in dark color and persons born abroad in light color. (The Public Health Agency of Sweden)



1.2 TUBERCULOSIS PATHOGENESIS

1.2.1 The pathogen

M.tb is a rod-shaped, slow-growing, aerobic and facultative intracellular bacteria. The mycobacterial envelope is thick and lipid-rich, consisting of long-chain mycolic acids, causing its acid fast property when microbiologically stained (17). The *M.tb* complex consists of *M.tb*, *M africanum*, *M bovis*, *M microti* and *M caprae*. TB-disease is usually caused by *M.tb* in humans. *M bovis* infection in humans can occur in contact with cattle or after BCG-vaccination or after BCG urine bladder instillation. The other members of the *M.tb* complex rarely cause disease in humans (18).

1.2.2 Transmission

The bacilli are spread by aerosols formed when an infected individual coughs, sneezes or sings. The aerosol contains droplets with one to three bacilli each. The droplets are small enough that when inhaled by another person, they reach the alveoli and can there establish infection (19). In an immunocompetent individual, immunity is usually established within 3 to 8 weeks after infection. The bacilli are then retained in a dormant stage, so-called latent tuberculosis infection (LTBI) (20). Endogenous reactivation of dormant *M.tb* in persons with LTBI (secondary or post-primary TB) occurs in 5-10% of infected individuals during their lifetime and usually happens within 2 years after the initial infection (20). In a person whose immune response does not control the primary infection, primary progressive TB develops. (21). If the new host develops pulmonary TB and *M.tb* can be detected in sputum, the circle of transmission is closed. Extrapulmonary disease is not contagious.

1.2.3 Immunity

In the upper respiratory tract, *M.tb* is encountered by the innate immune response in the respiratory mucosa. Local epithelial cells secrete mucus and antimicrobial peptides in an attempt to prevent the microbe from entering the deep airways. If the bacilli manage to penetrate this first defense barrier, they are ingested by alveolar macrophages that are the primary host cells to be infected with *M.tb*. Dendritic cells are essential in priming of naïve T cell responses, and thus uptake of bacterial products or apoptotic *M.tb*-infected macrophages are instrumental in triggering adaptive immunity. *M.tb*-infected macrophages and dendritic cells carrying *M.tb* antigens, migrate to regional lymph nodes and from there bacilli spread hematogenously further, throughout the body (22). *M.tb*-specific T cells are also primed in the lymph nodes and travel back to the site of infection in the lung to assist macrophages and other immune cells to combat the infection. This stage is known as primary TB infection and lasts from days to weeks; it is usually asymptomatic (22) but can sometimes cause transient disease symptoms, such as fever, erythema nodosum and poly arthritis (23).

In the macrophages the ingested bacilli are contained in phagosomes, where growth is restricted by acidification, reactive oxygen, nitric oxide and antimicrobial peptides (24). The intracellular bacteria can be killed by apoptosis of the macrophage (25) and the induction of autophagy (26) but complete eradication of *M.tb* is rare as the bacteria have developed strategies to survive these attacks (see below) (27). Infected macrophages secrete cytokines; interleukin (IL)-1b, IL-6, IL-12, IL-18 and tumor necrosis factor- α (TNF- α) which activate dendritic cells and attract T cells, natural killer (NK) cells as well as other immune cells subsets that result in enhanced local inflammation. Infected macrophages and dendritic cells present *M.tb* antigens on the cell surface via the major histocompatibility complex (MHC) class II. When recognized by CD4⁺ cells, differentiation and clonal expansion of T-helper 1 cells (Th1-cells) is induced. Th1 cells secrete IL-2, interferon- γ (IFN- γ) and TNF- α . TNF- α stimulates autophagy, enhancing

intracellular killing of *M.tb* in the macrophages, but also initiates cell migration and adhesion of new inflammatory cells and cell destruction within the infected tissue it affects (27).

Infected macrophages now fuse and start forming multinucleated giant cells or differentiate into foam cells. Infected macrophages attract lymphocytes, monocytes and neutrophils that surround the infected cells in a structure called a granuloma. The TB granuloma is an important hallmark of human TB. During progressive inflammation, apoptotic macrophages release bacilli into the center of the granuloma that can liquefy and support extracellular growth of *M.tb*. The center is first permissive of growth but as it becomes acidic and oxygen-depleted, *M.tb* bacilli turn into a dormant stage and only divide occasionally, so-called LTBI (28). Cytolytic CD8⁺ T cells (CTLs) also play an important role in the battle against *M.tb*. CTL cells secrete cytolytic (perforin and granzymes) and antimicrobial peptides (granulysin), killing the bacteria via granule-mediated cytotoxicity. However, excessive secretion of extracellular peptides leads to necrosis and tissue destruction. The CTL cell activity is downregulated by macrophages in the granuloma (29). During this phase antigen-specific long-lived memory T cells are formed, so called CD8 $\alpha\alpha$ ⁺ T cells, as they express the co-receptors $\alpha\alpha$ (30). Although *M.tb* is an intracellular pathogen and control of infection mainly dependent on cell-mediated immunity, the interest in humoral immunity has increased lately. B-cells have been shown to aggregate around granulomas. B cell depletion has been connected with hampered granuloma formation. Antibodies against lipoarabinomannan (LAM) and BCG have been shown to opsonize *M.tb* for phagocytosis by macrophages (31). Importantly, B cell can also have a role as antigen-presenting cells and may thus be involved in the activation of effector T cells.

In infants and immunocompromised individuals such as persons living with HIV or with impaired function of TNF- α or IFN- γ , granulomas are initially poorly formed and unstructured. This results in an early, enhanced dissemination of bacteria into the blood stream, a so-called primary progressive TB. If a person with LTBI is later immunocompromised, granulomas formed earlier lose their stability. The granuloma grows and a soft (caseous) necrotic center is formed. Bacilli can then escape into the bloodstream and airways (32).

M.tb has developed several strategies to survive in the infected individual. This is achieved by secreted effector molecules (33). In the mycobacterial genome, the region of difference 1 (RD1) encodes for the ESX-1 secretion system, producing the early secretory antigen target 6 (ESAT-6), culture filtrate protein 10 (CFP-10) and TB10.4, but also several other antigens. The antigens are secreted by the *M.tb* inside the macrophage and, by binding to the cell surface, help the bacilli translocate from the lysosome into the cytosol, thereby escaping degradation (34). The 85-antigen family (Ag85 a-c) has been found to prevent maturation of the lysosomes where the bacilli are contained (35). *M.tb* also uses other virulence mechanisms that promote spread to new cells and inhibit host cell apoptosis (32).

1.2.4 The role of HIV in TB pathogenesis

It is well known that HIV infection increases the risk of active TB, but also that TB increases HIV replication. Therefore, co-infection is advantageous to both pathogens, and has been called “the evil couple” or “the cursed duet”. People with untreated HIV are approximately 26 times more likely to develop active TB (36). The immune balance is lost as HIV proceeds. HIV infection leads to CD4⁺-cell depletion, reducing the body’s defense capabilities. When CD4⁺ cells are lacking, neutrophils are recruited in granulomas. Neutrophils induce IL-10 and IFN- α production, leading to further suppression of T cell function and *M.tb* growth (37). HIV also increases the numbers of CD8⁺ and CLT cells to control viremia. Unfortunately, these cells are dysfunctional because of exhaustion, with a low level cytotoxic peptides and a low TNF α production (29). HIV also infects alveolar macrophages, resulting in reduced macrophage viability, impaired *M.tb*-associated apoptosis inhibition of effector functions and accelerated

M.tb growth (38). Other parts of the immune system are also impaired by HIV co-infection (39), including down-regulation of MHC class II as well as co-stimulatory molecules on HIV-infected dendritic cells, which impairs priming of antigen-specific T cell responses. On the other hand, HIV entry into CD4⁺ cells and HIV replication are enhanced by *M.tb*-induced up-regulation of the HIV co-receptors CXCR4 and CCR5 (important for the entry of HIV virus into the CD4⁺ cell) as well as an induced pro-inflammatory cytokine cascade (39-42).

Patients with HIV and pulmonary TB have fewer necrotic granulomas and less pulmonary cavitation and therefore also lower transmissibility of *M.tb*. This can be explained by the fact that T cell responses to *M.tb* contribute substantially to cellular necrosis and tissue damage and with decreasing CD4⁺ cell numbers, these mechanisms are impaired (43).

Treatment of HIV with ART leads to recovery of the immune system. Early initiation has been proven to reduce mortality significantly (44). The numbers of CD4⁺ cells increases and central memory cells are redistributed from lymphoid tissues to the periphery. After three months of treatment a rise in naïve CD4⁺ cells and gradually also the level of effector cells is noted (45, 46). The recovering immune system regains its ability to react to the *M.tb* infection. The combination of antimycobacterial treatment and ART leads to rapid killing of bacilli. Large amounts of microbial components are released. This, in combination with regained and dysregulated immune response, can cause a so-called immune reconstitution inflammatory syndrome (IRIS). The patient, although actually recovering from both infections, presents with new or worsened clinical symptoms (39, 47). IRIS is caused either by worsening of known TB disease or unmasking of previously asymptomatic *M.tb* infection (48). The risk for IRIS is higher in patients with an initial low CD4⁺-count and high HIV-viral and bacillary load but also with short interval between TB treatment and ART introduction. In TB IRIS, an increased acute neutrophilic inflammation has been noted at the site of *M.tb* infection, before the recovery of the CD4⁺ T cells; this inflammation is interpreted as a recovery of the innate immune response and failure of immune regulation (49). The patient's genetic predisposition is probably also of importance for the development of IRIS (46). The overall estimated risk for IRIS is 18% with a mortality of about 3%, mainly in patients with central nervous system TB (47, 50). IRIS reaction has also been noted after discontinuation of TNF antagonist therapy (51).

If ART is introduced within six months after primary HIV infection, both CD4⁺ and CD8⁺ cells normalize. If treatment is delayed, the HIV infection turns into a chronic phase with a continuous high level of dysfunctional CD8⁺ cells and the CD4⁺ cells have an impaired IFN- γ production (29, 52).

1.2.5 TB antigens and vaccines

The BCG vaccine has been shown to induce clonal expansion of CD4⁺ and CD8⁺ cells that differentiate into effector memory T cells, migrating to the affected tissues, often the lung. Central memory T cells localize in secondary lymphoid organs. The cells can later proliferate and differentiate into new effector cells when exposed to *M.tb* antigen (53).

The RD1 genome (mentioned above) is missing in the less pathogenic BCG vaccine strains, *M. bovis* and in most environmental mycobacteria (54). In new, recombinant BCG vaccines the RD1 genome is reintroduced, rendering a more immunogenic but also a more virulent vaccine (55).

The new subunit or conjugate vaccines containing ESAT-6, CFP-10, TB10.4 and Antigen 85b (Ag85b), have been shown to induce IFN- γ secretion and to boost the central and resident memory CD8⁺ T cell and NK cell response achieved by BCG (55). Other interesting *M.tb* antigens used in the subunit vaccine candidate "M72/AS01E" are recombinant Mtb32A and Mtb39A (encoded by ppe18/Rv1196 and pepA/Rv0125). These antigens have been shown to bind to MHC class I and II epitopes and thereby induce CD4⁺ and CD8⁺ cell responses (56).

Other antigens (Rv2660c, Rv1733c, Rv1813c, Rv2628, Rv2029c, and Rv2659c) have been shown to be associated with latency and are used in vaccine candidates.

The immune responses measured in studies of different vaccine candidates, described below, are basically CD4⁺ and CD8⁺ cells and their expression of Th1 cytokines IFN- γ , TNF and IL-2. T-cells that produce a high amount of different cytokines are called multifunctional T-cells and have been shown to be crucial in determining protection of conjugate vaccines against a wide spectrum of pathogens. Durable immunity, has been shown to be obtained with the development of effector memory T-cells from multifunctional T-cells, mainly CD8⁺ cells. CD8 $\alpha\alpha$ ⁺ T cells has been shown to represent a compartment of long-lived memory T-cells. (57, 58). There is at present no immunological correlate between the magnitude of memory T cell response and their cytokine co-expression and protection against *M.tb* (59).

1.2.6 Drug resistance

During suboptimal treatment, resistant *M.tb* strains appear by the selection of pre-existing bacteria with random mutations for resistance (60). Drug-resistant TB (DR-TB) is often divided into: mono-drug-resistant – resistance to one first-line TB-drug; multi-drug-resistant (MDR)-TB – resistance to RIF and INH; extensively resistant (XDR)-TB – MDR resistance plus resistance to fluoroquinolone and any injectable drug (likely to change as injectables have been downgraded in treatment recommendations); and totally drug-resistant (TDR)-TB – resistance to a wider range of drugs than XDR-TB (14, 61).

1.3 TB INFECTION

TB infection is commonly divided into latent and active disease, but this seems to be a simplification. The current proposed paradigm is a dynamic spectrum ranging from full immunity to active TB disease (62). Despite the immune defenses described above, the bacilli manage to survive and continue replicating in the majority of cases. Active mycobacterial replication may eventually decline, leading at least temporarily to subclinical active infection. However, if the immune control is lost, for some reason, the bacterial load increases and symptoms and overt clinical disease develop (63). This concept is supported by the fact that isoniazid (INH), globally the most frequently used drug for the treatment of LTBI, acts by inhibiting mycobacterial cell wall synthesis and is therefore only efficacious against actively replicating organisms and that persons without clinical symptoms can be temporarily culture-positive for *M.tb* in sputum (64). This is also supported by the fact that the same mutation frequency (0.2-0.3 single nucleotide polymorphisms per genome per year (see below)) has been found in *M.tb* strains from patients infected decades before active disease as seen in outbreak strains (65). Despite these findings, I will in my presentation keep to the conventional latent and active TB concept.

1.4 LATENT TB INFECTION

LTBI diagnosis is based on immune recognition of TB antigens, as the numbers of bacilli are too small for identification. This means that the true prevalence of the disease is unknown and the sensitivity and specificity of the commercially available tests cannot be ascertained. The diagnosis of active TB is therefore often a surrogate marker of a former LTBI (66). Available tests for LTBI cannot differentiate between active disease, remote or recent LTBI or memory of previous infection (67). The overall global prevalence of LTBI in 2014 has been estimated to 23%, with a mathematical model (68).

1.4.1 Diagnosis of LTBI

Tuberculin skin test (TST): Tuberculin, first invented by Robert Koch, consisted of cultured, filtrated and heat-sterilized *M.tb* (69). It was later precipitated to isolated proteins in a standardized procedure, by Florence Seibert in the 1930s, forming the denoted Purified Protein Derivative (PPD-S) (70). The currently used PPD RT23 has been produced by Statens Serum Institute in Copenhagen since 1958. The dose of PPD is expressed in tuberculin units (TU) correlating to 0.02 µg of dry protein substance. The WHO recommends a dose of 2 TU (71). With the commonly used Mantoux-method, PPD is injected intradermally on the forearm. If the antigens are recognized the innate immune response activates dendritic cells and Langerhans cells. Antigenic material is phagocytized and presented to T cells. Secreted IFN-γ, TNF-α and IL-1 attracts neutrophils. Cellular infiltration causes a skin induration. This is called a delayed-type hypersensitivity (DTH) reaction (72). The transverse diameter of induration is measured after 48-72 hours and is expressed in millimeters. TST ≥5 mm is regarded as positive. This limit is used in Sweden, for non BCG-vaccinated children and immunocompromised patients. The TST reaction can be false positive due to cross-reactivity with non-tuberculosis mycobacteria (NTM) and BCG vaccination. Therefore, to improve specificity, the limit for a positive test is raised to ≥10 mm in BCG-vaccinated children and non-immunocompromised adults (73). The test can remain positive as a sign of retained immunoreactivity after cleared infection. This has been shown after completed treatment of active TB and is hypothetically transferable to LTBI (74). The test can also be false negative, see below. Other disadvantages of the test are the subjective nature of the assessment and measuring of the skin reaction, but also that the patient has to attend the clinic twice. Nonetheless, the test is well established and used worldwide.

Interferon gamma release assays (IGRAs): This relatively new technique is an *in-vitro* assay, invented to overcome the problem of cross-reactivity when using TST, described above. The *M.tb* secretory antigens (absent in BCG and most environmental mycobacteria), ESAT-6, CFP-10 and TB7.7, are presented to T cells. In case of recognition, IFN-γ production is induced and measured in the test. Two IGRAs are commercially available. In the QuantiFERON-TB Gold Plus (QFT) (Cellestis Limited, Carnegie, Victoria, Australia), whole blood is collected in four tubes, one containing ESAT-6, CFP-10 and TB7.7, stimulating CD4⁺ T cells, one with unknown antigens stimulating CD4⁺ and CD8⁺ T cells, one positive control containing mitogen and one negative control without stimulant. The IFN-γ production is measured using an enzyme-linked immunosorbent assay (ELISA) method (75, 76). In the T-SPOT.TB-test an enzyme-linked immunospot (ELISPOT) (Oxford Immunotec) method is used to detect lymphocyte-derived IFN-γ response to ESAT-6 and CFP-10. There is some evidence that the T-SPOT.TB test is more robust than the QFT in immunocompromised persons with low lymphocyte count, as a standardized number of cells per assay is used.

The tests for latent TB can all be false negative due to, for example, viral or bacterial infection (HIV, measles, mumps, typhoid fever, etc.), vaccination (other than BCG), disseminated TB, chronic renal failure, disease of lymphoid organs and medical immunosuppression (77).

In a review of 72 earlier studies in high and low TB endemic countries, the pooled sensitivity of TST (with 10 mm cut-off) and IGRA to detect LTBI in a non-BCG-vaccinated population was equally good (79%) and specificity was high (97%). In a BCG-vaccinated population the TST specificity was 59% (78). In a meta-analysis of 38 articles, the positive predictive value (PPV), to predict the risk of developing active TB among those with LTBI, were similarly poor for both TST and IGRA (TST 1–7% and IGRA 0–13%) (79). The negative predictive value of the tests is high (>99%) (80).

Flow-cytometric Assay for Specific Cell-mediated Immune-response in Activated whole blood assay (FASCIA): In this method whole blood is cultured with specific antigens for seven days. Thereafter, the sample is examined with a flow cytometric technique based on the differentiation, i.e. size and granularity, of resting and proliferating CD4⁺ cells and their cytokine response. The long-term incubation with antigens results in a larger number of responding cells than in QFT and T-spot TB test, when the cells are only incubated overnight. Mononuclear antibodies can be used for the detection of surface antigens in T cells subsets (CD3⁺, CD3⁺CD4⁺, CD3⁺CD8αβ⁺ and CD3⁺ 71 CD8αα⁺ T cells). Antigens used in TB diagnosis are Ag85A, 69 Ag85B and TB10.4 (81).

New diagnostic methods for LTBI: Gene expression analysis or so-called transcriptomics is a newly discovered and promising tool using genome analysis to measure RNA expression. Whole blood signature reflects changes in immune cell composition and altered gene expression for the discrimination of latent and active TB (82).

1.4.2 Treatment of latent TB

Screening and treatment for LTBI are important tools to achieve the WHO goals of reducing TB incidence (36). Screening for LTBI is recommended by both the Public Health Agency of Sweden and the WHO, for persons with high risk for later active TB. This includes newly arrived asylum seekers and persons with evidence of TB infection less than two years ago (with priority to children <18 years and pregnant women) and persons with untreated HIV infection, planned transplant-related immunosuppressive therapy or treatment with TNF-α inhibitors. Treatment is recommended to be offered to these groups if they show evidence or suspicion of LTBI, when active TB has been ruled out (83). Other factors with a high risk for disease are silicosis, chronic renal failure (hemodialysis) and fibronodular scarring of the lungs (83, 84).

The Public Health Agency of Sweden recommendations for the treatment of LTBI in adults (83):

- INH for 6-9 months, or
- INH and RIF for 3 months or
- Rifapentine and high dose INH once weekly for 3 months or
- RIF for 4 months (in case of known INH resistance)

These treatments have been shown to be effective and safe in several studies (85). The calculation of effectiveness is based on an estimated sensitivity of the test, treatment efficacy and adherence to treatment. Adherence to treatment regimens is a problem. In a systematic review of studies of LTBI treatment, initiation and completion in the European Union varied between 7-86% in migrants but was higher in patients with co-morbidities (75-92%) (86). In Sweden, the treatment completion rate in 2007 was on average 87% (87). In earlier randomized studies of HIV-negative patients with good adherence, nine months of INH was 90% protective against active TB (88). RIF alone or in combination with INH was even more effective (89). Hepatotoxicity is a feared adverse event (AE) associated with LTBI treatment. In adult persons without HIV infection treated with INH, hepatotoxicity appears in 5%, but in only 0.2-1.5% of patients treated with RIF alone or in combination with INH (88). The combination of INH and rifapentine has been shown to have a preventive effect similar to that of INH monotherapy but with fewer AEs and higher completion rates (90). LTBI treatment has been found to be cost-effective in selected groups in low-endemic countries (RIF and rifapentine-containing regimens are even more cost-effective than INH alone) (84, 91). The numbers of persons needed to screen (NNS) and treat (NNT) for LTBI to prevent one case of active TB can be calculated over a certain time (usually 5 years). The NNS and NNT values in migrants in the UK and Norway have been shown to be generally high and vary substantially with the rate of TB in the region of origin (92, 93). The implication of this is that screening and treatment

should be restricted to persons with additional risk factors: young age, recent TB contact and immunosuppression.

There is still little knowledge about LTBI treatment of patients exposed to MDR TB. Fluoroquinolones alone or in combination with ethambutol and/or with pyrazinamide have been tried. High treatment discontinuation rates due to AEs in persons taking pyrazinamide-containing regimens have been described. No regimen has yet been fully evaluated, but randomized trials are ongoing (94).

1.4.3 Latent TB and HIV

Early introduction of ART is vital for the prevention of active TB in people living with HIV. Since 2014 the recommendation has been to start treatment at HIV diagnosis (44, 95). The WHO recommends treatment of LTBI in patients with HIV (2). Overall the intervention rate has been low worldwide. The WHO estimates that out of 30 million people living with HIV, fewer than 1 million individuals are treated for LTBI (36), mainly because of fear of AEs, but also for concern about the development of drug-resistant tuberculosis. Several studies in high incidence countries have shown that INH for six to nine months, or INH combined with rifabutin or rifapentine for three months, reduces TB death and active TB considerably, regardless of ART, also in patients who test negative for LTBI (85, 96). INH treatment did not cause more serious AEs than placebo, and three months of INH and rifapentine was as safe as nine months of INH (90, 97, 98). LTBI treatment has not been shown to select resistant TB strains (99). Rifapentine alone for one month has recently been compared to nine months of INH in persons living with HIV in countries with a TB prevalence of >60/100,000 inhabitants. Rifapentine treatment was shown not to be inferior to INH for the prevention of active TB and TB mortality. The rifapentine group had a lower incidence of AEs and were more likely to complete treatment (100).

Screening and treatment for LTBI in persons living with HIV has been shown to be effective and safe also in low-endemic settings and is recommended not only by the WHO but also by the European Centre for Disease Prevention and Control (ECDC) and the American Center for Disease Control and prevention (CDC) (101-103). The Public Health Agency of Sweden recommends LTBI treatment for patients with HIV, primarily those who have not yet started ART or who have low CD4⁺ cell counts, once active TB has been ruled out (83).

Recommended treatment (depending on individual circumstances and concomitant medications, see below) (104):

1. Daily INH with pyridoxine for 6-9 months
2. Daily INH with pyridoxine and RIF/rifabutin for 3 months
3. Once-weekly isoniazid and rifapentine for 3 months
4. Daily RIF/rifabutin for 3 months

The National Reference Group for Antiretroviral therapy in Sweden recommends neither screening nor treatment for LTBI in people infected with HIV, as the mandatory HIV monitoring system is considered sufficient for early detection and treatment of active TB (95).

1.5 ACTIVE TB

1.5.1 Immunocompetent persons

Active TB is a disease that occurs in someone infected with *M.tb*. It is characterized by signs or symptoms of active disease. It can appear either directly after infection, when it is called primary TB, or more often after the awakening of dormant bacilli, also called secondary TB (105). In the vast majority of cases, TB activation occurs within two years after primary infection (106). The most common form is pulmonary TB ranging from 70-90% in different settings (107). Symptoms of pulmonary TB are cough, fever, night sweats, weight loss and sometimes hemoptysis, dyspnea and chest pain. The infection is often localized in the apical segment of the lung lobe and starts as bronchiolitis. Mediastinal lymph node enlargement and pleural effusion are also common at an early stage. Later, cavitation and sometimes extensive lung destruction appears. (105). Extrapulmonary TB is a result of hematogenous dissemination. Either bacilli that have spread during primary infection, rest dormant and awaken, causing local infection, or bacteria disseminate during post-primary infection. Extrapulmonary TB can occur in all parts of the body but is most common in intrathoracic and cervical lymph nodes. Other common forms are pleuritis, bone and joint infection, genitourinary and intestinal TB, and central nervous system infection (108). (Miliary TB is described below). Persons from the African and Asian continents have been shown to be generally more likely to present with extrapulmonary manifestations than Europeans, and females are also overrepresented in this group. Genetic factors may explain some of these differences (109).

1.5.2 Immunocompromised persons

HIV infection gradually leads to CD4⁺-cell depletion and increased risk for both primary active TB and reactivation of latent infection. With a sinking CD4⁺ cell count, in untreated HIV infection, the ability to generate solid granulomas decreases and hematogenous spread of *M.tb* and disseminated, extrapulmonary disease becomes more common. Numerous small granulomas are formed in different organs. The radiological picture resembles millet seed, with 1-to-4-mm rounded seed-like opacities, giving it the name miliary TB. Disseminated TB is a serious condition often manifested as pleuritis, pericarditis and meningitis with only vague and nonspecific symptoms such as chronic fever. In a meta-analysis of earlier studies from high- and low-incidence countries, the mortality rate in TB during TB treatment was 19% in persons living with HIV but 4% in persons without HIV (110). Advanced pretreatment immunodeficiency persistently increases the risk of TB, also after the introduction of ART (111). A higher risk of TB also remains in patients treated with ART but with ongoing HIV replication (112).

Other forms of medical immunosuppression and immunocompromising diseases also affect the TB incidence. Patients treated with TNF antagonist therapy have up to 25 times higher risk of active TB (113), and those undergoing solid organ transplantation, 20-75 times higher risk (114), but also chronic corticosteroid treatment, chronic renal failure and hemodialysis, hematological malignancies and diabetes mellitus are known risk factors for TB activation (21).

1.5.3 Diagnosis of active TB

TB is diagnosed with a combination of microbiology, radiography and sometimes histopathology. Microbiological diagnosis of pulmonary TB consists of *smear microscopy*, *polymerase chain reaction (PCR)* and *culture* of sputum samples. In the absence of sputum production, samples from bronchial or gastric lavage are commonly used in high resource settings (108). HIV testing should be recommended to all patients with TB (115).

Sputum-smear microscopy is most commonly performed with Ziel-Neelsen staining using the acid-fast property of the bacteria. *M.tb* cannot be differentiated from NTM with this technique. The test is a cheap and simple and has been used worldwide since its invention by Robert Koch in 1882. It has since been improved with sputum sample centrifugation, auramine or rhodamine staining and fluorescent microscopy with LED light, used for analysis. A positive test requires at least one bacillus in a minimum of one examination of at least 100 microscopic fields, in one sputum sample from a TB suspect. The test has a low sensitivity compared to culture (25-75%) and a positive test requires 5000 bacilli per ml of sputum (116). Sputum smear-positive patients are seen as highly contagious (108).

Polymerase chain reaction (PCR) is a line probe assay technique used to amplify certain DNA sequences. With this method, *M.tb* infection can rapidly be identified in smear-negative but later culture-positive patients. PCR has an acceptable sensitivity for sputum samples but it is inferior for other samples, especially fluids (cerebrospinal and pleural fluid) (117, 118). The PCR method should not be used for treatment control as dead bacteria can cause false positive results (119). The method can distinguish *M.tb* from most NTM-infections and can also be used to detect common mutations in genes coding for drug resistance (120). The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is a point-of-care test for the detection of *M.tb* and signs of RIF resistance in sputum, giving a result within two hours. The method has replaced smear microscopy in many low-income settings. It improves diagnostic accuracy by 23% compared to microscopy alone, among culture-confirmed cases (121).

In *Culture*, *M.tb* grows slowly, with a generation time of 18–24 hours. A specimen that shows no evidence of growth after 8 weeks is classed as negative. The gold standard for TB diagnosis is culture of mycobacteria, performed on solid egg-based (mostly Löwenstein-Jensen) and agar-based media in parallel with broth-based liquid media. The advantage of solid media is their ability to reveal slow-growing bacteria, mixed cultures and contaminants. The combination of the two diagnostic culture methods renders fast and more accurate diagnosis, with about 80% sensitivity (122). After identification of mycobacterial growth, a chromatographic immunoassay or line probe assay is used to discriminate between *M.tb* complex and NTM. Conversion from positive to negative sputum culture within two months from treatment initiation is used as a sign of effective treatment in pulmonary TB. If this is achieved, relapse and failure are unlikely (123). However, sputum samples are often hard to obtain after two months, as many patients have improved significantly.

Adenosine deaminase is an enzyme required for the proliferation and differentiation of T lymphocytes and the maturation of monocytes to macrophages. The enzyme is elevated in diseases associated with cellular immunity and is widely distributed in tissues and body fluid. The test is used as a diagnostic aid preferably in TB meningitis, pericarditis, peritonitis and pleuritis (124).

Another diagnostic method involves detection of *mycobacterial lipoarabinomannan (LAM) antigen*. Lipopolysaccharide present in mycobacterial cell walls is released from metabolically active or degenerating bacterial cells. The antigen can be detected in urine from patients with active disease. In persons living with HIV with CD4⁺ cells ≤ 100 cells/ μ l, a positive urinary LAM test has high specificity (but a low sensitivity) for active TB. A positive test has also been shown to be associated with a high mortality in this group. As a substantial proportion of these patients have low sputum bacillary load, the point-of-care urine LAM test can be used as complement to sputum microscopy (125). Much effort is being made to further explore the LAM test for the diagnosis of active TB but also as a predictive biomarker of the outcomes of TB treatment and for the evaluation of treatment efficacy (126).

Alternative biomarkers to improve immune diagnosis of TB and monitoring of treatment efficacy are under development. Promising results have been achieved with the chemokine,

IFN- γ -induced protein 10 (IP-10), produced by antigen presenting cells in patients with TB, irrespective of HIV status. IP-10 seems to be higher in patients with active TB than LTBI and decreases under active TB treatment. It might in the future be used to monitor therapy efficacy (81, 127).

Radiography: Radiography is an important tool in the diagnosis of TB. In suspicion of pulmonary TB chest radiography (x-ray) can detect shadowing, caverns and enlarged mediastinal lymph nodes. Computer tomography (CT) scan is more sensitive than chest x-ray and detects minor abnormalities such as bronchiolitis (“tree-in-bud” phenomenon) and miliary TB. CT scan is also used for the diagnosis of extrapulmonary TB (18). Positron emission tomography (PET) CT is a relatively new tool used for the detection of active TB but can also assess therapy response. It measures the uptake of injected ^{18}F -fluorodeoxyglucose (^{18}F -FDG), in inflammatory cells. The site of infection is visualized with a CT scan (128).

Histopathology: In microscopy, TB infection is visualized as granulomatous inflammation with aggregated macrophages, epithelioid cells and multinucleated giant cells formed around a necrotic center (18). The pattern is less characteristic in immunocompromised patients, for instance those with HIV co-infection.

Detection of resistance: Culture-based phenotypic drug susceptibility testing on solid or in liquid media, is the standard method. It can detect and assess the degree of resistance to both first and second line drugs (129). As the method is slow (requiring 1-4 weeks), new methods have been invented the recent decades. PCR-based molecular techniques for rapid detection of gene mutations related to resistance are in use. The following genes are associated with drug resistance to the most used drugs in TB-treatment: RIF - *rpoB*; INH - *katG/inhA/ahpC*; fluoroquinolones - *gyrA*; injectable antibiotics (capreomycin, kanamycin, amikacin) – *rrs*; ethambutol – *embB*; and pyrazinamide - *pncA* (130). The semi-automated Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) detects mutations in the genetic region of *rpoB* (indicating multi-drug resistance) in sputum, within 2 hours, with a high sensitivity and specificity (121). Whole Genome Sequencing (WGS) is a precise method for genotypic drug resistance analysis which has high concordance (95-96%) with culture-based methods (131, 132). WGS is cheaper and faster (9 days) than culture-based methods but is only available at specialist centers (133). However it is important to remember that the relationship between mutations and phenotypic resistance is not completely known and they do not always overlap. In about 10% of phenotypically INH-resistant strains no mutation in either *katG* och *inhA* is found, indicating an existence of so far unknown mutations and resistance mechanisms. On the other hand mutations that are not expressed does not lead to resistance (130).

1.5.4 Treatment of active TB (not pregnant, adults)

TB must be treated with a combination of drugs, as monotherapy selects resistant *M.tb* subpopulations (134). Adherences to treatment is crucial for cure. Support and treatment supervision must be individualized to each patient. Directly observed treatment (DOT) or video-observed treatment is one option, but other forms acceptable to the patient and to the health system can be used (135). In Sweden drugs are often distributed in dosing boxes for 2 weeks’ use, refilled at the TB clinic.

1.5.4.1 Treatment of drug-sensitive TB

TB treatment is designed to kill different subpopulations of *M.tb* isolates. Fast-replicating bacteria are rapidly killed by the bactericidal INH, but to eradicate slow-replicating bacteria, RIF and pyrazinamide are needed (136). The treatment regimen for sensitive *M.tb* infection is a combination of INH, RIF, pyrazinamide and ethambutol during the intensive phase for two months, followed by a four-month consolidation phase with RIF and INH (137). Ethambutol

is added to reduce the risk for treatment failure until susceptibility has been confirmed. In patients with TB meningitis, the treatment should be prolonged to 9-12 months, and in case of bone infection to 9 months (137). Appropriate serum level of anti-mycobacterial drugs is of major importance for treatment success and can be measured for most drugs. Corticosteroid treatment should be added in case of TB in the central nervous system.

RIF, INH and pyrazinamide are metabolized by the cytochrome P450 enzyme system in the liver, rendering their potential hepatotoxic effect. Genetic polymorphisms affecting this system has been show to increase the risk of liver injury in certain populations (138). RIF is a potent inducer of the enzyme system affecting metabolism and thereby the systemic concentration of other drugs. This must always be considered when starting TB treatment in patients with polypharmacy. Rifabutin is a less potent inducer of the cytochrome P450 enzyme system and can therefore replace RIF in combination with ART (see below), warfarin and calcineurin inhibitors, among other drugs (139).

1.5.4.2 Treatment of resistant TB

In patients with confirmed RIF-susceptible and INH-resistant tuberculosis, treatment with RIF, ethambutol, pyrazinamide and levofloxacin is recommended for a duration of 6 months (140).

In patients infected with *M.tb* resistance to one first line drug, other than INH, treatment is prolonged to 12-18 months and ethambutol or a fluoroquinolone replaces the inactive substance (137).

Globally most MDR-TB patients are recommended a total treatment duration of 18-20 months or 15-17 months after sputum culture conversion. In recently published WHO recommendations, a 9-12-month regimen could be considered if resistance to fluoroquinolones and second-line injectable agents has been excluded (140). In high-income countries such as Sweden, the resistance pattern is often known and the treatment regimen can be suited thereafter. Shortened, all-oral, bedaquiline-containing treatment courses are under evaluation in trials (STREAM II) (141). The regimen should contain drugs presented in **Table 1**. All Group A agents and at least one Group B agent should be included to ensure that treatment starts with at least four TB agents likely to be effective, and at least three agents should be included for the rest of the treatment after bedaquiline is stopped (bedaquiline can be used only for six months). If only one or two Group A agents are used, both Group B agents are to be included. If the regimen cannot be composed with agents from Groups A and B alone, Group C agents should be added (140). Treatment monitoring and patient support is even more important for patients with MDR TB

ART is recommended for all patients with HIV and drug-resistant TB requiring second-line TB drugs, irrespective of CD4⁺ cell count and as early as possible (within the first 8 weeks) following initiation of anti-TB treatment (140).

Table 1. Recommended drugs for MDR-TB treatment – 2019 WHO update

Group	Drug	Adverse reaction
A (include all if possible)	Moxifloxacin/Levofloxacin	Arthralgia, achilles tendon rupture, polyneuropathy
	Bedaquiline	QT-prolongation, liver toxicity
	Linezolid	Pancytopenia, polyneuropathy
B (add both if possible)	Clofazimine	Reversible skin discoloration
	Cycloserine OR Therizidone	Neurotoxicity, psychiatric disturbances, neuropathy
C (add when drugs from group A and B cannot be used)	Ethambutol, Delamanide, Pyrazinamide, Imipenem-cilastatin, OR Meropenem/clavulanic acid, Amikacin, Ethionamide/Prothionamide, <i>p</i> -aminosalicylic acid	

1.5.4.3 Adverse reactions

Hepatotoxicity is the most common severe reaction with an incidence ranging between 2-30% in different populations (142-144). The incidence is higher in elderly, in Asian populations, in persons living with HIV, chronic viral hepatitis or with concomitant alcohol abuse or use of other drugs (143, 145). Hepatotoxicity is defined as elevated alanine aminotransferase (ALT) level to ≥ 3 times the upper limit of normal in the presence of hepatitis symptoms, or ≥ 5 times the upper limit of normal in the absence of symptoms. Patients should be closely monitored (137).

Other common side effects are listed in **Table 2**. Some of them can be severe while others are disturbing but often tolerable with symptomatic treatment (137). To avoid neuropathy, pyridoxine is administered to patients treated with INH. For early detection of optic neuritis caused by ethambutol, vision and color perception are tested monthly in Sweden (146).

Table 2. Adverse reaction of first-line TB drugs

Drug	Adverse reaction
Rifampicin	Rash, nausea, fever, hepatotoxicity, cytopenia, allergy, shock, acute renal failure
Isoniazid	Vertigo, nausea, headache, neuropathy, allergy, hepatotoxicity, depression
Pyrazinamide	Vertigo, nausea, hepatotoxicity, arthralgia, allergy, hyperuremic syndrome
Ethambutol	Hyperuremic syndrom, optic neuritis

Serious AEs are more common with MDR treatment and appear in 17% with linezolid and in 2% bedaquiline (140). The most common AEs are listed in **Table 1**.

1.5.5 Treatment outcome

According to the WHO the estimated TB cure rate for the 5.9 million new TB cases in the 2016 global cohort was 82% (9). The death rate after completed TB treatment has been shown to be 3.8 times higher than in the general population in low-endemic, high-income settings (147).

Pulmonary TB is regarded as cured if sputum is smear- or culture-negative the last month of treatment. If sputum samples cannot be produced, completed treatment is regarded as

successful by the WHO (137). Monitoring patients after completion of treatment is not recommended in the WHO guidelines, except for one-year post-treatment monitoring of drug-resistant TB (14). In Sweden TB patients are followed for TB relapse for 6-12 months after treatment completion, and longer in case of resistant TB.

Recurrent TB is defined as episodes of TB occurring in a person after treatment completion and can be caused either by endogenous infection with the same strain (relapse), or by reinfection with a different strain (reinfection) (148). The relapse rate in low-endemic settings has been shown to be 0.4-7% (149-151). Genotyping is used to separate relapse from reinfection. Risk factors for recurrent infection have been shown to include male sex, low socioeconomic status, origin from a high-endemic region, diabetes mellitus, smoking, alcohol abuse, intravenous drug use, infection with a Beijing lineage strain, MDR TB and pulmonary cavitation and CD4⁺ cell depletion in persons living with HIV (152).

1.6 GENOTYPING OF *MYCOBACTERIUM TUBERCULOSIS*

Molecular typing of *M.tb* isolates detects disease transmission and clusters of TB cases and is an important part of the TB control strategy. Drug resistant *M.tb* isolates have been genotyped in Sweden since 1994 and drug-susceptible strains have also been included since 1998. The initial method was restriction fragment length polymorphism (RFLP) (153, 154) combined with spoligotyping (155). In 2012 the genotyping method was changed to the faster, mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) method (156) combined with spoligotyping. These methods analyze and compare only standardized parts of the genome.

Since 2016, WGS has replaced the methods previously used in Sweden. WGS has a higher discriminatory power than RFLP and MIRU-VNTR (157) and is as fast as MIRU-VNTR, generating a result within a month. With WGS, the genetic similarity between strains is measured in the numbers of single nucleotide polymorphisms (SNPs). *M.tb* has been reported to have a high genomic stability with a steady genetic turnover rate of around 0.3-0.5 SNPs per genome per year (158, 159). The SNP threshold, to define a TB cluster in epidemiologically linked cases and after recent transmission, has been established to a maximum of five SNP differences (132, 160). The use of WGS offers quicker contact tracing and more precise cluster investigations, which is important to limit transmission of the disease (157, 161). Efforts are being made to harmonize the nomenclature of WGS by the use of a reference strain comparing specific loci (160).

In contact investigation, the connection between individuals is described in a minimum spanning tree. The degree of genetic dissimilarity between the bacterial strains they are infected with is used to link together the individuals that carry the most similar strains, indicating a possible transmission (162).

WGS can also be used to distinguish relapse with the same *M.tb* strain from reinfection with a new strain in patients previously treated for TB. With earlier used techniques, reinfection was considered the main cause of recurrent infection in high-endemic countries (163, 164). In contrast, relapse was considered more frequent in low-endemic regions (152). As WGS has a higher resolution than previously used methods, some cases regarded as relapse based on the older genotyping methods, would be regarded as reinfection based on WGS results (157). Also, WGS has the capacity to better identify minority *M.tb* populations, as in infections with several strains, so-called mixed primary infection (165, 166).

As mentioned, WGS can be used to identify mutations coding for drug resistance.

1.7 TB-VACCINES

1.7.1 BCG

The BCG vaccine is weakened attenuated strains of *Mycobacterium bovis*, administered with an intradermal injection. It was developed at the Pasteur Institute in Lille, France in 1921. Continuous passaging has led to several substrains currently being in use. The strain divergence has not led to any significant variation in vaccine efficacy (167). BCG has shown an over 80% efficacy in preventing serious forms of disease, such as meningeal TB in children under the age of five (168, 169), but in adults the efficacy ranged from zero to 80% in different populations and its protective effect appears to decline with time (6). The effect of the vaccine varies with the geographic location. The protective effect has been shown to be stronger at higher latitudes, but closer to the equator the effect seems weaker (170). A placebo-controlled study conducted among native Americans in the 1930s showed significant protection against active TB up to 40 years after BCG vaccination (171). More recent studies from the northern hemisphere confirm a vaccine effectiveness of 50-60% for 20 years and still 40% for another 20-40 years (172, 173). Differences in the protective effect in various countries could be explained by the differences in initial protection. In TB-endemic areas, infection with *M.tb* prior to BCG vaccination may render a weaker immune response to the vaccine and therefore a lower protection rate (167). Continued and extensive exposure to NMT in warmer countries is another explanation for reduced BCG reactivity (174).

The current global recommendation from WHO is to administer BCG vaccination at birth in TB-endemic countries (175). In countries with low rates of TB, selective vaccination is used under continuous disease surveillance (176). The Public Health Agency of Sweden does not recommend BCG vaccination of adults but only of children who come from a TB-endemic area, have a close relative with active TB, or who will be traveling for more than three months to or in TB-endemic areas where contact with locals is expected (177). Revaccination with BCG is not recommended, but recent studies have shown some evidence of reduced risk of *M.tb* infection after BCG revaccination (178). As BCG is a live vaccine, it is contraindicated in immunocompromised persons, such as people living with HIV, who are at heightened risk of hematogenous dissemination (176).

1.7.2 New vaccines

Three basic approaches for new TB vaccines are:

- preventive, pre-exposure vaccines or priming vaccines, which are administered to neonates, prior to the first exposure to *M.tb*. The primary concept is to find a live vaccine to replace BCG, but subunit vaccines are also in clinical trials.
- preventive post-exposure vaccines or boosting vaccines which are targeted to adolescents and adults with LTBI and prior BCG immunization. Inactivated live vaccines and subunit vaccines are in clinical trials.
- therapeutic vaccines, administered with regular TB drugs.

1.7.2.1 Live vaccines

Inactivated whole-cell vaccine derived from a NTM is used as a boost vaccine for BCG-vaccinated individuals. It has been shown to be safe, immunogenic and effective in phase 1, 2 and 3 studies in TB endemic areas and has advanced to efficacy trials (179).

Recombinant BCG (rBCG) has been developed both as a replacement for BCG vaccination in infants and as a TB vaccine in adolescents and adults. BCG strains are engineered by the insertion of virulence factors from other bacteria. For example in the VPM1002-vaccine

candidate a gene, derived from the bacteria *Listeria Monocytogenes*, coding for listeriolysin O, has been inserted. Listeriolysin O perforate the lysosome, permitting the recombinant strain to escape from the macrophage lysosome into the cytosol and thereby the macrophage overexpress antigens which improve CD8⁺ T cell induction. It also induce autophagy and apoptosis (180). This vaccine candidate has been shown to be more effective in preventing active TB but less virulent than the original strains (55). It has been tried and proven safe in a phase 2 trial in HIV-infected and HIV-uninfected infants. A phase 3 trial is now being conducted in infants, and a phase 2b/3, randomized, double-blind, placebo-controlled trial is being conducted to assess the efficacy in preventing recurrence of TB in adults recently treated for and cured of active TB (181, 182).

Live attenuated M.tb vaccines MTBVAC have been developed primarily to replace BCG as a priming immunization against TB in infants and has passed phase 1 trial. These *M.tb* strains have genetically manipulated genes coding for virulence factors permitting its survival in host cells. MTBVAC is also being assessed in safety studies, for use in previously BCG vaccinated adolescents and adults (181, 182).

Alternative BCG strategies: in rhesus macaques, aerosol or intravenous administration of BCG has been shown to better induce a mycobacterium-specific immune response and reduce disease severity compared to intradermal injection (183).

1.7.2.2 Subunit vaccines

Subunit vaccines have been developed to boost BCG-derived immune response to provide a long period of protection. They are composed of several TB antigens that are considered immunogenic and protective (ESAT-6, CFP-10, TB10.4, Ag85A, Ag85B, Mtb32A, Mtb39A, and Rv2660c), combined with an adjuvant or a viral vector for increased immunogenicity. They are well tolerated and induce a sustained immune response in humans (184-186).

In *viral vectored vaccines* *M.tb* antigens are combined with modified and attenuated vaccinia virus (pox virus), adenovirus or influenza virus for enhanced uptake and efficacy. The vaccine candidate MVA85A uses vaccinia as a vector, combined with Ag85A, and has been shown to be safe and immunogenic in a phase 2 trial but showed no significant efficacy against active TB in previously BCG vaccinated infants (187). As prime vaccination in HIV-exposed newborn children it induced a modest antigen-specific immune response (188).

Recombinant cytomegalovirus vectors encoding *M.tb* antigen have been tested in animal models (189).

In *protein-adjuvant vaccines*, a fusion of *M.tb* TB antigens is combined with an adjuvant (IC31, CAF01, AS01 or GLA-SE) that prolong antigen exposure and enhancing the uptake of antigens into antigen presenting cells and thereby stimulating immunity (190).

The M72/AS01E vaccine consists of the antigens Mtb39A and Mtb32A combined with the adjuvant AS01E. The vaccine is intended to prevent TB in adults and adolescents. It has a clinically acceptable safety profile both in non-HIV-infected and HIV-infected individuals (191, 192). Recently the ability of the M72/AS01E vaccine to provide protection against active pulmonary TB disease in previously TB infected individuals (QFT-positive) was assessed in a randomized, double-blinded, placebo-controlled study (193). The trial was conducted on 3,330 HIV-negative adults in TB-endemic countries. The vaccine provided 54% protection against active pulmonary TB-disease during 2.3 years of follow-up. No serious AEs were reported but more injection-site reactions and influenza-like symptoms were seen with active vaccine compared to placebo.

The H4:IC31 vaccine candidate consists of the antigens TB10.4 and Ag85B combined with the IC31 adjuvant and has been shown to be safe and immunogenic in phase 1 trials (184, 185). The vaccine was included in a phase 2 study in South Africa, published 2018 (178). Almost 1,000 QFT-negative young adults were vaccinated twice with either H4:IC31, BCG or placebo and followed regularly for QFT conversion for 24 months. Neither vaccine prevented initial QFT conversion but both vaccines showed a significantly reduced rate of sustained QFT conversion. The sustained conversion was 8.1% in the H4:IC31 group and 6.7% in the BCG group, as compared with 11.6% in the placebo group. These findings may reflect a reduction of sustained *M.tb* infection. There were no clinically significant differences in the rates of serious adverse events between the groups.

H56:IC31 is a vaccine composed of Ag85B, ESAT-6 and Rv2660c with the IC31 adjuvant. It has been developed to prevent TB disease at all ages and to prevent recurrent TB. Phase 2 trials testing its ability to serve as adjunctive immunotherapy and thereby shortening the treatment duration for active TB are ongoing (182).

Studies employ different protocols and methods to measure Th1-cytokine expression and the entailed level of protection is uncertain, which makes comparisons problematic. With that caveat, it can be noted that the M72/AS01E induced the highest memory CD4⁺ T cell response (59). Overall, a better correlate of protective TB immunity would facilitate assessment of disease prevention and also promote consistency in immunogenicity measures comparing different trials.

1.7.2.3 Therapeutic vaccines

The therapeutic vaccines are used as immunotherapy and are thus not regular vaccines but rather therapy adjuncts.

Heat-inactivated *M vaccae*, available in injectable and oral forms, has been shown to shorten the time to sputum conversion and radiographic resolution in patients with pulmonary TB by activating the Th1 cytokine-mediated immune response and macrophage phagocytosis. It has been shown effective as adjuvant therapy with general chemotherapy for the treatment of MDR-TB and has been approved for this purpose for patients in China (194).

A phase 2 trial with aerosol administration of NTM (*M. indicus pranii*) as an adjunct to TB treatment is ongoing (195).

RUTI is composed of detoxified, inactivated, latency-associated *M.tb* antigens. These antigens are hidden during the active phase of TB and therefore not recognized by the immune system. After immunization with RUTI the immune system also attacks dormant bacilli. The vaccine has been tried for the prevention of active TB. Given as a subcutaneous injection after a total of one month of LTBI treatment, it has been shown to induce a specific cellular but also a strong humoral immune response. It has been shown safe in phase 2 trials, including patients living with HIV (189).

2 AIMS

2.1 GENERAL AIMS

The general aim of this thesis is to increase the knowledge of TB and especially TB-HIV co-infection, with an emphasis towards prevention, in a low-endemic setting in an area of increasing migration from TB-endemic countries.

2.2 SPECIFIC AIMS

2.2.1 Paper I

To identify factors associated with anti-TB treatment success as well as adverse drug reaction in HIV-TB-co-infected persons in the era of ART.

2.2.2 Paper II

To test the safety and immunogenicity of the new TB vaccine in a prime-boost vaccination strategy in healthy, previously BCG-vaccinated individuals.

2.2.3 Paper III

To understand the usefulness of chemoprophylaxis among people living with HIV in a low-endemic setting today.

2.2.4 Paper IV

To evaluate current treatment strategies and treatment control in Stockholm County by analyzing of the respective frequency of relapse and reinfection.

3 MATERIALS AND METHODS

3.1 STUDY SUBJECTS AND METHODS

3.1.1 Paper I

3.1.1.1 *Setting and data collection*

All adult patients (aged ≥ 18 years) diagnosed with both HIV and TB in Stockholm County, 1987-2010 were identified in the Swedish national quality registry named InfCare HIV. TB diagnosis was defined as symptoms and laboratory findings leading to TB treatment. The criteria for TB diagnosis was fulfilled in 127 patients. In 109 patients (86%), TB was confirmed. In the remaining 18 patients, diagnosis was based on PCR, clinical, radiological, or histopathological signs suggestive of TB which responded to treatment. Data related to demographic characteristics and HIV/AIDS (acquired immunodeficiency syndrome) status were collected from InfCare HIV, the Swedish TB registry (containing data on all cases of active TB in Sweden) and from medical records.

3.1.1.2 *Definitions*

- The clinical manifestation of TB was defined as:
 - “Isolated pulmonary” if TB involved only the lungs.
 - “Extrapulmonary” if TB was diagnosed in organs other than the lungs, including lymph nodes and pleura.
 - “Disseminated” if at least 1 extrapulmonary site and the lungs were involved.
- Anti-TB treatment was classified as first-line or second-line, based on the choice of drugs according to the WHO guidelines (137).
- Antiretroviral treatment was classified as ART if consisting of NRTIs combined with a PI, non-nucleoside reverse-transcriptase inhibitors (NNRTI), or fusion inhibitor (FI); or at least 3 NRTIs according to the Swedish recommendation for the treatment of HIV infection in 2009 (196).
- Adverse reactions were defined as severe, noxious or unintended responses to anti-TB treatment that led to treatment discontinuation or modification, based on the judgment of the attending physician.
- Outcomes of anti-TB treatment were defined as:
 - “Successful” for patients who were cured or completed treatment without any registered TB recurrence within the follow-up period until the end of 2011.
 - “Unsuccessful” in the case of death due to the primary diagnosed TB infection or death irrespective of reason during the TB treatment period, or discontinued treatment.
 - “Not evaluated” in the case of transfer to another clinic before treatment completion.

3.1.1.3 *Study design*

Because of the introduction of ART in 1996 the cohort was stratified into two cohorts: the early cohort (1987-1995) and the late cohort (1996-2010). Sociodemographic and clinical characteristics were compared for the two cohorts. ART was evaluated as possible predictor of a successful anti-TB-treatment and of adverse reactions.

3.1.2 Paper II

A new TB vaccine candidate, H4:IC31 composed of the H4 antigen which is a fusion protein created from two *M.tb* antigens Ag85B and TB10.4, and an immunological adjuvant called IC31® was evaluated in two phase 1 randomized, double-blind, placebo-controlled studies , in Stockholm and in Finland.

3.1.2.1 Inclusion criteria

Previously BCG-vaccinated (≥ 5 years), males or females (females required to be sterilized), age 18-50 years, HIV-uninfected, good health based on medical history, normal body mass index (19-33), no evidence of an ongoing TB infection and written informed consent completed.

Both studies employed vaccine dose escalation, with increasing amounts of the H4 antigen administered in the presence of increasing amounts of the IC31 adjuvant **Table 3**. Formulation buffer was used as placebo control. Subjects received vaccinations via intramuscular injection on study days 0 and 56. Treatment assignments were based on a randomly generated sequence of subject identification numbers on a randomization schedule.

Table 3. A dose matrix of the H4 and IC31 dose combinations administered to the study subjects in the C-005-404 and C-006-404 trials*

H4 dose	One vaccination		Two vaccinations				Total (n=124)
	Day 0			Day 0 and 56			
	50 µg	150 µg	5 µg	15 µg	50 µg	150 µg	
No adjuvant	8	8	---	---	8	---	24
100 nmol IC31	8	---	9	9	8	---	34
500 nmol IC31	8	---	8	8	16**	8	48
Placebo	---		18***				18

* A total of 124 participants were included in the two trials. **Two doses of 50 µg H4 in 500 nmol IC31 were administered to 8 participants in each of the C-005-404 and C-006-404 trials. ***A total of 18 participants received two placebo vaccinations in the two trials.

3.1.2.2 Adverse events

Safety evaluation of the H4:IC31 vaccine treatment regimens was based on the induction of AEs that represented both clinical and laboratory evaluations. We recorded solicited AEs (derived from the study subject's diary, from physical examination and laboratory results) and unsolicited AEs (spontaneously reported by the subject) during the first 28 days after each vaccination and serious AEs (SAEs) during the 6-month study period.

AE severity was graded according to the US Food and Drug Administration (197) as:

- Mild (Grade 1), no medical intervention required
- Moderate (Grade 2), requiring outpatient medical intervention
- Severe (Grade 3), requiring prolonged medical intervention and/or hospitalization
- Potentially life-threatening (Grade 4), requiring hospitalization with intensive care

3.1.2.3 Immunogenicity testing

Subjects were screened for ongoing TB infection using QFT and the TST. Peripheral blood mononuclear cells (PBMC) were isolated from blood collected on study days 0, 7, 14, 28, 56, 63, 70, 84, and 182 for intracellular cytokine staining (ICS) and on study days 0, 56, 84, and 182 for assessment of *M.tb*-specific IFN- γ production using ELISpot. In study C-005-404, blood was also collected on study days 0, 7, 28, 63, 84, and 182 for Flow-cytometric Assay for Specific Cell-mediated Immune-response in Activated whole blood assay (FASCIA).

3.1.3 Paper III

3.1.3.1 Study population

The study included all adult patients (aged ≥ 18 years) diagnosed with HIV at Karolinska University Hospital in Stockholm, from January 1996 through December 2013. The patients were identified in InfCare HIV, and data on; sex, year of birth, route of HIV transmission, country of origin, date of HIV diagnosis, HIV viral load and CD4⁺ count at HIV diagnosis, CD4⁺ nadir (during the study period) and, where applicable, date of death, we extracted from the registry. For patients who were diagnosed with TB during the study period additional data were extracted: date of TB diagnosis, CD4⁺ count and HIV RNA viral load at TB diagnosis, date for ART initiation and ART regimen. Information on TB manifestation, diagnostic procedures and treatment regimens was retrieved from the national reporting system and registry, SmiNet and medical records.

The patient cohort was divided into two groups, based on the year of HIV diagnosis (1996-2008 and 2009-2013) due to the changed guideline recommendations for the introduction of ART in 2009. During the first part of the study period (1996-2008), guidelines recommended ART for patients with a CD4⁺ count of 250 cells/ μ l or less; in the later part of the study period (2009-2013), guidelines recommended ART for patients with a CD4⁺ count under 350 cells/ μ l.

3.1.3.2 Definitions of terms and concepts

- Active TB was defined as a specimen positive for acid fast bacilli in smear microscopy or PCR or culture for *M.tb* or if radiography or histology was characteristic for active TB and led to treatment for active TB.
- TB diagnosed within one month from HIV diagnosis was assumed not to have been preventable with LTBI-treatment and was defined as *early TB*. In contrast, TB diagnosed more than one month after HIV diagnosis was considered to have been potentially preventable with LTBI-treatment and was defined as *late TB* in coherence with previous studies (198, 199). Patients were considered to be at risk of preventable TB from one month after HIV diagnosis until TB diagnosis or death or end of follow-up.
- Country of origin was classified as TB-endemic versus low-endemic, based on the WHO estimates of TB incidence in different countries (36). Based on these estimates, countries in sub-Saharan Africa and Asia were classified as TB-endemic and all other countries as low-endemic.

- Clinical manifestation of TB was defined as *isolated pulmonary* if only the lungs were affected; *extrapulmonary* if other organs than the lungs were affected, including the pleura and thoracic lymph nodes, or *disseminated* if the lungs and at least one extrapulmonary site were involved.
- In patients from TB-endemic countries, *latent TB* was considered possible in absence of regular TST or IGRA test results.
- CD4⁺ nadir was defined as the lowest CD4⁺ value measured within the study period.
- ART was defined as a combination therapy involving two NRTIs plus one of the following: a PI, a NNRTI, or a fusion inhibitor (FI).
- ART was classified as: *possibly effective* if ART had been administered for more than 3 consecutive months; or *confirmed effective* if ART had been administered for more than 3 consecutive months and HIV-RNA was undetectable (<50 copies/ml) (95).

3.1.4 Paper IV

3.1.4.1 Setting and data collection

All TB cases diagnosed in Stockholm County, from January 1996 through December 2016, were identified in the national reporting system and SmiNet-registry. Only cases with culture confirmed *M.tb* infection were eligible for inclusion, and were followed for TB recurrence until the end of 2017. Demographic and clinical data and microbiological results were also extracted from the registry. For patients with recurrent TB, information on risk factors for recurrence was retrieved from individual patients' files. Information on HIV co-infection was obtained from the Swedish national quality registry (InfCare HIV). WGS was performed on the *M.tb* strains in cases with TB recurrence to distinguish relapse from reinfection, and SNP variants were compared between the TB episodes.

3.1.4.2 Definitions

- A case of TB recurrence was defined as a patient, previously successfully treated for TB, who had a new TB episode more than 180 days after treatment completion.
- Treatment success was defined as cure or treatment completion according to the WHO definitions (200, 201).
- The anatomical site of TB disease was defined as pulmonary, extrapulmonary or disseminated according to the WHO definitions (201).
- The patient's country of origin was regarded as endemic if it had a TB incidence of $\geq 100/100,000$ population and as low endemic if $< 100/100,000$ (9).
- A relapse case was defined as a maximum difference of five SNPs.
- *M.tb* resistance was defined as mono-drug resistance or MDR resistance, according to the WHO definitions (200, 202).

3.1.4.3 DNA extraction, whole genome sequencing and detection of resistance mutations

DNA was extracted from *M.tb* strains and subsequently quantified (153, 203). The sequencing reads were compared with a reference genome. The similarity between isolates from the same patient was studied by SNP analysis. A distance matrix was calculated and the relatedness was visualized in a minimum spanning tree (162). In order to detect resistance mutations, the sequencing reads were mapped against a set of resistance genes (*rpoB*, *katG*, *inhA*, *embB*, *pncA*, *gyrA*, *gyrB*, *rrs*, *eis*, *tlyA* and *ethA*) derived from a reference genome.

4 STATISTICAL ANALYSIS

In general a p-value of <0.05 was considered significant for all studies and all tests were two-sided.

Descriptive statistics were used to analyze sociodemographic and clinical data and are presented as medians and interquartile ranges (IQR) for numeric variables and as percentages for categorical variables. Comparisons between groups were performed using the Mann-Whitney *U*-test for continuous variables. A Chi-square test, or where appropriate Fisher's exact test, was used for categorical variables. **Study I, III and IV.**

In **Study I and IV** we used logistic regression analysis to estimate associations of patient characteristics with outcome variables. In **Study III** a Cox proportion hazard regression model was used to analyze risk factors for active TB over time.

In **Study III** the incidence rates were calculated as the annual number of patients diagnosed with TB divided by the annual number of person-years at risk. Trends in incidence rates were calculated using a Poisson regression model presented as percent change in incidence rate per year.

In **Study III** the number needed to treat (NNT) for latent TB, to avoid one case of active TB, was calculated as the reciprocal of the incidence of TB (the number of TB cases/the number of patients at risk for TB).

In **Study II** the sample size for each trial was calculated as adequate for the assessment of the safety profile. Basic descriptive analyses were used to examine AEs and immune response for each treatment regimen and area under the curve (AUC) analyses were made for comparison of the different immune responses to different treatment regimens. Overall p-values for median AUC among treatment regimens were obtained using the Kruskal-Wallis test. Pairwise comparisons of median AUC between treatment regimens were conducted using a Mann-Whitney exact test. The Holm method was used for correction of multiple comparisons, reducing the risk of type I error.

5 RESULTS AND DISCUSSION

5.1 PAPER I

5.1.1 Results

The study included 127 patients with HIV and TB co-infection, of whom 26 were stratified into the early cohort (1987–1995) and 101 into the late cohort (1996–2010). A majority of the patients were originally from a country other than Sweden, mainly from sub-Saharan African countries (85% in the early cohort and 88% in the late cohort). There was a significant increase in female patients from 26% ($n=6$) in the early cohort to 49% ($n=49$) in the late cohort ($p=0.02$). Over 40% of patients in both cohorts were diagnosed with TB within 6 months from their HIV diagnosis.

5.1.1.1 Antiretroviral treatment

In the early cohort none of the patients received antiretroviral treatment classified as ART but 13 (50%) were treated with single or dual NRTIs.

In the late cohort, 76/101 patients (76%) were treated with ART during the TB treatment period. ART was initiated before TB-diagnosis in 31 patients. A PI-based regime was used in 53 patients. The percentage of patients who received ART was highest among those with disseminated TB, 90% (35 of 39), compared to 64% (14 of 22) among those with extrapulmonary TB and 68% (27 of 40) among those with isolated pulmonary TB ($p = 0.026$).

5.1.1.2 Anti-TB treatment

The percentage of patients who completed their anti-TB treatment successfully increased from 65% in the early cohort to 91% in the late cohort ($p=0.002$). Sixteen patients (62%) in the early cohort and 77 patients (76%) in the late cohort received anti-TB treatment with first-line TB drugs. Three patients (12%) in the early cohort and 10 patients (10%) in the late cohort had single-drug resistant *M.tb* strains and 2 patients (2%) in the late cohort had MDR TB. In non-MDR TB cases the drug regimen was based on RIF in 58 patients (59%) and rifabutin in 41 patients (41%). Of those on RIF, 21 patients (36%) switched to rifabutin on initiation of ART. The median duration of successful anti-TB treatment among patients with drug-susceptible TB ($n=94$), excluding 6 patients with TB in the central nervous system, was 8 months (IQR 6 – 27) in the early cohort ($n=11$) and 8 months (IQR 6 – 10) in the late cohort ($n=77$).

5.1.1.3 Adverse reactions to anti-TB treatment in the late cohort

Out of 99 patients in the late cohort whose treatment outcomes were evaluated, 23 (23%) suffered from adverse reactions severe enough to lead to modification of anti-TB treatment. Twenty-two (96%) of these patients were on concomitant ART, **Figure 5**: 3 patients were on ART at the time of TB diagnosis and 19 initiated ART at a median 2 months (IQR 0.7 – 3.5) after TB diagnosis. All 23 patients with adverse reactions completed treatment successfully after a median 11 months (IQR 9.0 – 13.5). The reported adverse reaction manifestations were: affected liver function ($n=8$; 2 of whom had chronic hepatitis C), neurological complications ($n=5$), IRIS ($n=3$), affected vision ($n=3$), rash ($n=3$), renal complications ($n=1$), joint pain ($n=1$), gastrointestinal complications ($n=1$), and other complications ($n=2$).

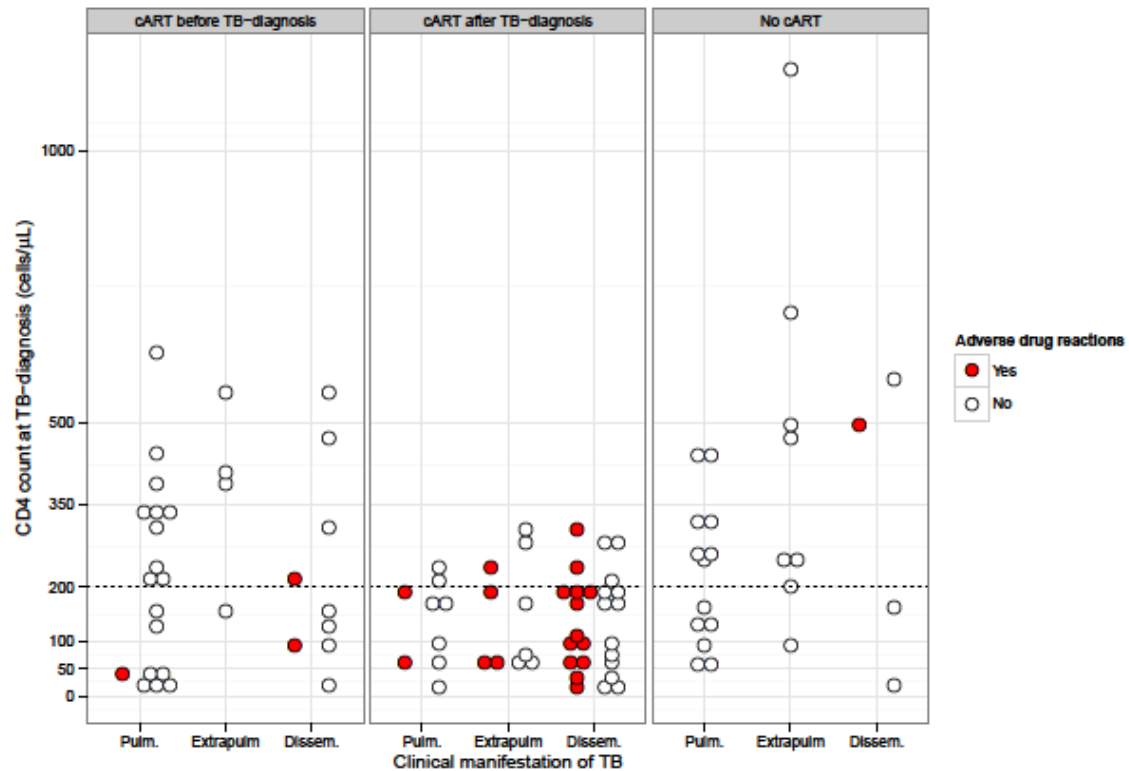


Figure 5. Dot plot representing the occurrence/non-occurrence of adverse reactions to anti-TB treatment in relation to the CD4 cell count (cells/ μ l) at the time of TB diagnosis among HIV-infected patients diagnosed with TB 1996 or later ($n=99$). Three groups are compared. The dotted horizontal line represents the cut-off level for immunosuppression, which was specified at $CD4 \leq 200$ cells/ μ l.

5.1.1.4 Predictors of treatment success and adverse reactions in the late cohort

In the multivariate logistic regression analysis (**Table 4**), treatment success in the late cohort was positively associated with ART (OR 13.3, 95% CI 1.5 – 114.8; $p=0.018$) and a $CD4^+$ cell count >200 cells/ μ l (OR 17.2, 95% CI 1.2 – 236.6; $p=0.034$). The occurrence of adverse reactions in the late cohort was positively associated with the initiation of ART after TB diagnosis (OR 13.3, 95% CI 1.6 – 112.4; $p=0.018$).

Table 4. Results of logistic regression analysis: factors related to treatment success and adverse reactions to anti-TB treatment for patients with HIV and TB, diagnosed 1996 or later (n=99).

Variables	Successful treatment outcomes (n=99)			
	Yes (n=92)	No (n=7)	Bivariate analysis	Multivariate analysis
	n (%)	n (%)	OR (95% CI)	OR (95% CI)
ART				
Yes	72 (78)	3 (43)	4.8 (1.0-23.2)	13.3 (1.5-114.8)*
No	20 (22)	4 (57)	Reference	
Sex				
Female	47 (51)	1 (14)	6.3 (0.7-54.1)	5.9 (0.5-71.1)
Male	45 (49)	6 (86)	Reference	
Age at TB diagnosis, y				
≤37	57 (62)	1 (14)	9.8 (1.1-84.6)*	9.8 (0.9-105.0)
>37	35 (38)	6 (86)	Reference	
CD4 ⁺ count at TB diagnosis, cells/μl				
≤200	51 (55)	6 (86)	Reference	
>200	41 (45)	1 (14)	4.8 (0.6-41.7)	17.2 (1.2-236.6)*
Adverse reactions to anti-TB treatment (n=99)				
Variables	Yes (n=23)	No (n=76)	Bivariate analysis	Multivariate analysis
	n (%)	n (%)	OR (95% CI)	OR (95% CI)
ART				
ART before TB diagnosis	3 (13)	27 (36)	2.6 (0.2-26.3)	2.8 (0.3-29.3)
ART after TB diagnosis	19 (83)	26 (34)	16.8 (2.1-135.6)**	13.3 (1.6-112.4)*
No ART	1 (4)	23 (30)	Reference	
Sex				
Female	13 (57)	35 (46)	1.5 (0.6-3.9)	1.0 (0.3-3.0)
Male	10 (44)	41 (54)	Reference	
Age at TB diagnosis, y				
≤37	18 (78)	40 (53)	3.2 (1.1-9.6)*	2.7 (0.8-9.0)
>37	5 (22)	36 (47)	Reference	
CD4 ⁺ count at TB diagnosis, cells/μl				
≤200	18 (78)	39 (51)	3.4 (1.2-10.1)*	1.8 (0.5-6.2)
>200	5 (22)	37 (47)	Reference	

5.1.2 Discussion

This study confirms what is well known worldwide: that the introduction of ART in 1996 thoroughly changed life for persons living with HIV and so also the outcome of TB treatment in this group. In low-endemic settings, such as Stockholm, TB is largely a disease affecting persons from TB-endemic countries. Changes in the immigrating population are therefore reflected in the group of patients diagnosed with TB. The increasing proportion of women in the late cohort was accounted for by patients migrating from sub-Saharan Africa and Asia.

We observed that most cases of adverse reactions occurred in patients who initiated ART after TB diagnosis, especially patients with a low baseline CD4⁺ cell count. The association was not statistically significant in a multivariate regression analysis but has been shown to be related in earlier studies. As expected the most frequently noted adverse reactions were effects on liver function and neurological complications. The vast majority of patients in our late cohort were treated with PI in combination with two NRTIs combined with a rifabutin-based TB regime, which can have influenced the incidence and panorama of AEs. Current national Swedish national guidelines (as of 2018) for treatment of HIV, recommend an efavirenz-based regimen in combination with RIF or an integrase inhibitor (INI)-based regimen with rifabutin (204). The INI regimens have fewer AEs compared to both PI and efavirenz, and the changed recommendations may have entailed an improvement for HIV and TB co-infected individuals (205, 206).

Our study might have underestimated the frequency of IRIS. Some of the patients' symptoms regarded as AEs can very well have been IRIS. Our definition of IRIS was that the physicians in charge note IRIS as an explanation for the patients' symptoms in medical records. There might be a tendency to underreport IRIS as the cause of symptoms, as there are no consensus definitions for IRIS.

5.2 PAPER II

5.2.1 Results

We enrolled 125 individuals and randomized them into the different intervention groups. In the Swedish study, all 64 randomized subjects received the vaccination on study day 0 and all completed the study. In the Finnish study, 60 of 61 randomized subjects received the vaccination on study day 0 and all subjects except two completed the study.

5.2.1.1 Adverse events

The majority (83%) of subjects across both studies had AEs graded as mild or moderate. **Table 5A and B.** Severe AEs were noted in 18 (15%) subjects of which four were considered related to the study vaccine: fever, increased protein in urine, TST site reaction, and increased international normalized ratio (INR). The overall incidence and severity of the AEs did not differ between active vaccine and placebo. Four SAEs were reported [mental status changes (50/0 one dose regimen), mesenteric lymphadenitis (50/500 two dose regimen), ileus (50/500 two dose regimen) and subdural hemorrhage (placebo)], none of which was considered related to study vaccination.

Table 5A. Adverse Events by Highest Severity for Each Subject: C-005-404 (Swedish study).

Severity	H4:IC31 (µg H4/nmol IC31)							
	Placebo (n=8) n (%)	50/0		50/100		50/500		150/0
		1 Dose (n=8) n (%)	2 Doses (n=8) n (%)	1 Dose (n=8) n (%)	2 Doses (n=8) n (%)	1 Dose (n=8) n (%)	2 Doses (n=8) n (%)	1 Dose (n=8) n (%)
Mild	3 (37.5)	4 (50.0)	2 (25.0)	4 (50.0)	2 (25.0)	1 (12.5)	2 (25.0)	1 (12.5)
Moderate	4 (50.0)	2 (25.0)	5 (62.5)	2 (25.0)	4 (50.0)	6 (75.0)	4 (50.0)	4 (50.0)
Severe	1 (12.5)	1 (12.5)	1 (12.5)	2 (25.0)	2 (25.0)	1 (12.5)	2 (25.0)	3 (37.5)

Table 5B. Adverse Events by Highest Severity for Each Subject: C-006-404 (Finnish study).

Severity	H4:IC31 (µg H4/nmol IC31)						
	Placebo 2 Doses (n=10) n (%)	5/100	5/500	15/100	15/500	50/500	150/500
		2 Doses (n=9) n (%)	2 Doses (n=8) n (%)	2 Doses (n=9) n (%)	2 Doses (n=8) n (%)	2 Doses (n=8) n (%)	2 Doses (n=8) n (%)
Mild	4 (40.0)	3 (33.3)	4 (50.0)	3 (33.3)	2 (25.5)	1 (12.5)	—
Moderate	5 (50.0)	6 (66.7)	3 (37.5)	5 (55.6)	5 (62.5)	6 (75.0)	6 (75.0)
Severe	—	—	1 (12.5)	1 (11.1)	1 (12.5)	—	2 (25.0)

Among solicited AEs, myalgia, arthralgia, and fever occurred at a higher frequency in subjects who received the active vaccine compared to placebo. Pain at the injection site was increased in subjects who received IC31 adjuvant. Among the first 21 included (all TST negative) subjects in the Swedish study, 14 experienced some degree of inflammation at the site of screening TST injection, after the first vaccination. The reactions were elicited by the H4 antigen alone (78.6%) or H4 combined with a low dose of IC31 adjuvant (21.4%). One subject experienced a severe reaction at the TST site with onset the day of the first study vaccine administration. This subject had no screening TST reactivity or a reaction at the vaccine site.

5.2.1.2 Immunogenicity

In each of the H4:IC31 two dose regimens, the study vaccine induced Ag85B-specific but also TB10.4-specific CD4⁺ T cell responses, with a boosting effect seen after administration of the second dose. The strongest and most long-lived median CD4⁺ T cell responses compared to placebo were seen after stimulation with Ag85B in the 5/500 (p<0.01), 15/500, and 50/500 (p<0.01) two-dose regimens. These results were supported by the whole blood FASCIA assay, where the two-dose 50/500 treatment was shown to induce significant expansion of Ag85B-specific CD4⁺ T cells (p=0.02) and CD8αα⁺ T cells (p<0.001) compared to the two-dose placebo regimen. IFN-γ ELISpot responses to both Ag85B and TB10.4 were elevated, but only for the two-dose regimens together with the IC31 adjuvant. **Figure 6.** ICS analysis of Ag85B-specific CD4⁺ T cells at 4 weeks after the second vaccination (study day 84) confirmed that only the two-dose treatment regimens efficiently induced cytokine

producing cells, primarily bi-functional IL-2/TNF- α producing and multifunctional IFN- γ /IL-2/TNF- α producing T cells.

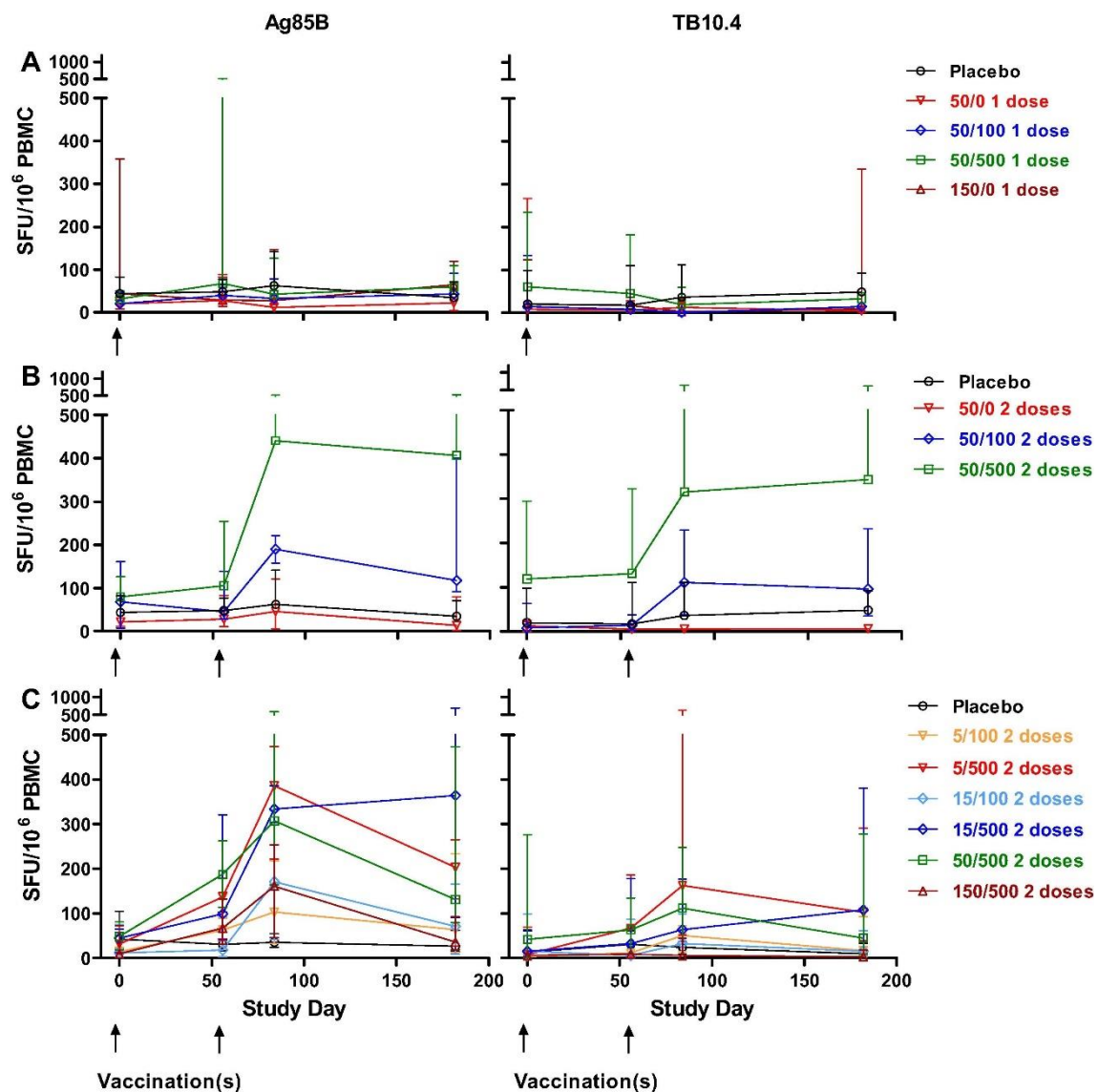


Figure 6. H4:IC31 vaccination induces antigen-specific IFN- γ production in peripheral T cells from BCG-vaccinated study subjects. Frequencies of Ag85B-specific IFN- γ producing T cells induced by the H4:IC31 vaccine were measured using the IFN- γ ELISpot assay after stimulation of PBMCs with *M.tb* antigen-peptide pools. Expression of IFN- γ in Ag85B-specific T cells is shown for the different treatment regimens at study days 0, 56, 84 and 182 in study C-005-404 one dose (A) and two dose (B) regimens and C-006-404 (C). The data illustrate medium-subtracted spot-forming cells per 10^6 PBMCs for all vaccinated subjects. Data are presented in linear graph plots showing the median and IQR for each group.

5.2.2 Discussion

This phase 1 study demonstrates that the H4:IC31 vaccine candidate is safe in healthy and previously BCG-vaccinated individuals. The local and systemic AEs were mild to moderate and did not increase in frequency or severity with a second vaccination. The highest H4 antigen dose (150 μ g) induces systemic, solicited AEs (myalgia, arthralgia and fever) at a higher frequency. The AEs resolved within three days. A high dose of IC31 adjuvant caused significantly more injection site reactions. These findings have been confirmed in later and

larger phase 1 and 2 studies in high-endemic settings (178, 207). A post-vaccination inflammatory reaction was observed at the TST injection site in some of the subjects screened with TST at inclusion. The reaction appeared mostly in patients given only H4, without adjuvant. This delayed hypersensitivity reaction is caused by PPD-specific T cells rapidly migrating to the TST site in response to vaccine antigens that are also present in PPD. A similar reaction was observed with another vaccine candidate (Mtb72F/AS02) (208). This reaction needs to be considered in future trials.

We could show that two doses of H4:IC31 vaccine induce a stronger CD4⁺ T cell, CD8αα⁺ T cell and IFN-γ response than a one-dose regimen or placebo. The effect was most pronounced after stimulation with Ag85B. These findings were later confirmed in a phase 1 study of the same vaccine candidate, in South Africa 2015 (207). A low dose of antigen combined with a high dose of adjuvants induced the strongest response. This may be explained by the fact that at a low dose, the antigen selectively induces a higher number of multifunctional T cells. This has earlier been associated with considerably stronger protection against subsequent *M.tb* challenge in mice (209).

The more recent study (described above) of the H4:IC31 vaccine, given in the dose; H4, 15 µg and IC31, 500 nmol, to former BCG vaccinated adolescents, in a TB high-endemic setting, showed a significant reduction of sustained QFT conversion. This result indicates that the vaccine candidate might render some degree of protection against *M.tb* (178). The results are encouraging for the future prospects of subunit TB vaccines but in its current formulation, it seems not be the ultimate solution for the global epidemic.

5.3 PAPER III

5.3.1 Results

5.3.1.1 Patient characteristics

During the study period 1,868 patients were followed from HIV-diagnosis until TB diagnosis or death or until the end of observation period. During the study period 92 patients (5%) in the cohort were diagnosed with TB. The median age at HIV diagnosis was 36 years (IQR, 29–44). The majority of the patients were men (58%). Most had acquired HIV through heterosexual transmission (65%) and were originally from TB-endemic countries (52%). Patients diagnosed with TB during the study period had a significantly lower median nadir CD4⁺ and median CD4⁺ at HIV diagnosis ($p<0.001$) and a significantly higher HIV viral load ($p<0.001$). Of the 92 patients who were diagnosed with TB, six patients (7%) died, whereof two from TB. The proportion of patients from TB-endemic regions increased significantly over the study period (1996-2001: 45%, 2002-2007: 53%, 2008-2013: 58%, test for trend: $p=0.004$).

5.3.1.2 Incidence and prevalence of TB

Overall incidence of TB

The 1,868 persons living with HIV were followed over 14,740 person years (PY), which corresponds to a median follow-up time of 7.9 (IQR, 3.9–11.5) years.

Of the 92 TB cases, 48 (52%) were diagnosed within one month after HIV diagnosis (early TB) and 44 (48%) were diagnosed more than one month after HIV diagnosis (late TB).

The overall incidence rate of TB during the study period was 6.2 (95% CI 5.1–7.6) per 1,000 PY. During the study period we observed a significant decrease in incidence of TB; this was equivalent to an annual rate decline of -10.1% [95% CI, -15.1-(-6.8)]. **Figure 7.**

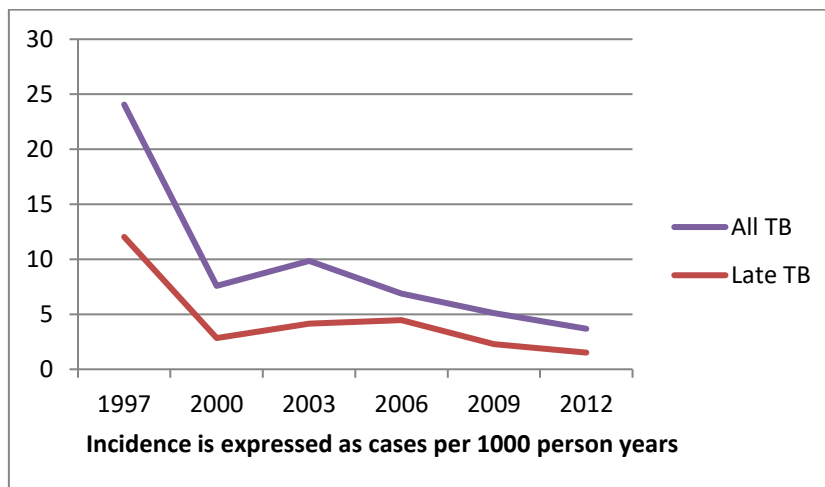


Figure 7. Incidence rate of TB in patients living with HIV in Stockholm county 1996-2013, divided into three-year periods. The upper curve represents the incidence rate of TB overall. The lower curve represent the incidence within the subset of patients diagnosed with late TB (diagnosed >1 month after HIV diagnosis).

The incidence of TB during the overall study period was 1.0 (95% CI 0.5-2.0) per 1,000 PY in patients from low TB-endemic regions and 11.3 (95% CI 9.0-14.0) per 1,000 PY in patients from TB-endemic regions.

Prevalence of early TB

We estimated the prevalence of TB at the time of HIV diagnosis under the assumption that patients diagnosed early with TB, i.e. within one month from their HIV diagnosis, represent prevalent rather than incident cases. The overall prevalence of early TB during the study period was 2.6% (95% CI 1.9-3.4). The prevalence of early TB increased over the study period: 1.6% (95% CI 0.6-3.9) during the first three years and 4.4% (95% CI 2.4-7.9) during the final three years (see Figure 3 in Paper III). However, the observed trend of an increasing prevalence of TB early at HIV diagnosis did not reach the level of statistical significance ($p=0.064$).

Incidence of late TB

The overall incidence of late TB was 3.0 per 1,000 PY (95% CI, 2.2–4.0). A significant decrease in the incidence rate of late TB was observed during the study period [-11.1% (95% CI, -16.9-(-5.0))]. **Figure 7.**

5.3.1.3 Characteristics of TB-patients

Of the 92 patients with HIV-TB co-infection, TB disease was culture-confirmed in 79 of the patients (86%). Three were diagnosed by PCR, one by sputum microscopy, five by radiography and three by histopathology. Resistant TB strains were detected in four patients, two had a multi-drug resistant strain. Active TB was diagnosed within one year from HIV diagnosis in

68 (73%) of the 92 co-infected patients. A TST or IGRA test was performed in ten of the 92 patients and was positive in six.

The characteristics of the HIV-TB co-infected patients (n=92) during the study period (grouped into early and late TB cases) are presented in. **Table 6.** Disseminated TB and lower median viral load at TB diagnosis were significantly more often seen among the 44 patients with late TB diagnosis compared to the 48 patients with early diagnosis.

Table 6. Characteristics of TB-patients with HIV (n=92) grouped into early and late TB.

	All TB patients (n=92)	Early TB (n=48)	Late TB (n=44)	Early vs Late TB <i>p</i> -value*
CD4 at TB diagnosis , (cells/μl) median (IQR)	160 (61-260)	168 (64-259)	154 (52-282)	0.759
HIV viral load at TB diagnosis (cop/ml) median (IQR)	90,100 (1,392-550,000)	346,000 (56,050-813,000)	10,295 (50-253,250)	0.045
Patients diagnosed per time period, n (%)				
1996-2008	64 (69%)	31 (65%)	33 (75%)	0.278
2009-2013	28 (31%)	17 (35%)	11 (25%)	
Time on ART at TB diagnosis, n (%)				
No ART	64 (70%)	37 (77%)	27 (61%)	
≤3 months	18 (20%)	11 (23%)	7 (16%)	
>3 months	10 (10%)	0	10 (23%)	
Continent of origin , n (%)				
TB-low endemic	10 (11%)	4 (8%)	6 (14%)	0.414
TB-endemic	82 (89%)	44 (92%)	38 (86%)	
Clinical manifestation of TB , n (%)				
Isolated pulmonary	38 (41%)	27 (56%)	11 (25%)	0.010
Extrapulmonary	22 (24%)	9 (19%)	13 (29%)	
Disseminated	32 (35%)	12 (25%)	20 (46%)	
Time from HIV to TB diagnosis , n (%)				
<1 months	48 (52%)	48 (100%)	-	
1 months – 1 year	20 (22%)	-	20 (46%)	
1-2 years	8 (9%)	-	8 (18%)	
2-6 years	15 (16%)	-	15 (34%)	
>6 years	1 (1%)	-	1 (2%)	

IQR, interquartile range. **p*-values refer to comparisons of the distribution of disease characteristics between patients with early versus late TB diagnosis. Statistically significant (*p*<0.05) differences in proportions of patient characteristics are bolded.

In the late TB group, 32 (73%) patients were ART naïve. Seven (16%) had had effective ART (more than three months of treatment and a viral load <50 copies/ml at the time of TB diagnosis).

5.3.1.4 Risk factors of late TB in the HIV cohort

An increased risk for active TB more than one month from HIV diagnosis was significantly associated with heterosexually transmitted HIV and origin from TB-endemic continent in a bivariate Cox regression analysis. After adjustment in the multivariate analysis, continent of origin was the only characteristic that had a statistically significant association with an increased risk of late TB. **Table 7.**

Table 7. Results from Cox regression analysis with active TB more than one month after HIV diagnosis as dependent variable by characteristics at HIV diagnosis in persons diagnosed with HIV in Stockholm County 1996-2013 (n=1795).

	HIV (n)	TB (n)	Bivariate analysis HR (95% CI)	p-value	Multivariate analysis HR (95% CI)	p-value
Total (n)	1,795	44				
Sex						
Male	1,051	22	1.39 (0.77–2.51)	0.272*	1.33 (0.70-2.51) ³	0.386
Female	744	22	Reference			
Age (years)						
<35	833	25	1.46 (0.80–2.65)	0.215*	1.00 (0.54–1.86)	0.999
≥35	962	19	Reference			
Route of HIV transmission						
Heterosexual	1,155	35	2.13 (1.02-4.43)	0.043†	1.04 (0.46-2.33)	0.920
Other	640	9	Reference			
Continent of origin						
TB endemic	873	39	7.54 (2.97-19.13)	<0.001†	8.54 (3.09-23.61)	<0.001†
TB low endemic	922	5	Reference			
Year of HIV diagnosis						
1996-2008	1,351	37	1.16 (0.51-2.64)	0.726*	Not included	
2009-2013	444	7	Reference			
CD4 at HIV diagnosis (cells/μl)						
<200	536	18	1.67 (0.91-3.04)	0.095*	Not included	
≥200	1,243	26	Reference			
VL at HIV diagnosis (cop/ml)						
<500	232	1	Reference	0.060*	Not included	
≥500	1,531	43	6.70 (0.92-48.68)			

HR, Hazard ratio; CI, confidence interval; VL, viral load. *Variables with a *p*-value >0.05 in bivariate analysis were not included in the multivariate analysis except for sex and age, which were considered to be potential confounding factors. †Statistically significant odds ratios (*p*-value < 0.05) are bolded.

5.3.1.5 Numbers needed to treat (NNT) to prevent one case of active TB

Based on the assumption that only the 44 patients (out of the 1795 HIV patients at risk of late TB) diagnosed with late TB could have had their disease prevented with LTBI treatment, and, anticipating that LTBI treatment would give full protection against active TB, 41 (95% CI, 31–56) patients would have to be treated with LTBI to prevent one case of active TB (during a median follow-up time of 7.4 (IQR, 3.9–11.5) years). In patients originating from TB-endemic regions the NNT was 22 (95% CI, 15–30) (during a median follow-up time of 6.7 (IQR, 3.5–11.1) years), also anticipating full protection by LTBI treatment.

5.3.2 Discussion

With the implementation of ART, we observed a significant decline in incidence rate of TB over time. The annual mean decrease was approximately 10%, which is in the same range as in most other contemporary studies from low TB incidence countries. To further reduce the frequency, the WHO recommends screening and treatment of LTBI in risk groups. To identify patients who could possibly benefit from LTBI treatment, we analyzed the association between certain patient characteristics at the time of HIV diagnosis and the risk of developing late TB. In this adjusted analysis, originating from a TB-endemic country was the only characteristic that was significantly associated with active TB development, a finding in congruence with other studies. Low CD4⁺-cell count and high HIV viral load at HIV diagnosis were also associated with late TB diagnosis in two contemporary studies from low-endemic settings but our study did not lend any support to a predictive role of these two characteristics for risk of developing late TB when modern ART was introduced early.

The majority (77%) of patients with potentially preventable TB (late TB) were ART naïve or had been treated with ART less than 3 months at time of TB diagnosis, which underscores the importance of the currently implemented strategy of introducing ART already at diagnosis in all patients diagnosed with HIV. Notwithstanding the probable protective effect of immunological reconstitution after ART, it is still worth noting that the incidence of late TB in our cohort at the end of the study period was roughly 80 times higher than that of the general population in Stockholm during the same time period according to the Public Health Agency. TB is still a potentially lethal infection in persons living with HIV.

The current WHO guidelines recommend screening for and treatment of LTBI in patients diagnosed with HIV in low-endemic countries. This strategy is also supported by the Public Health Agency of Sweden. This policy is supported by a Swiss study from 2007 in which a significant decrease in subsequent TB incidence was shown after the implementation of a LTBI screening program (210). However, current WHO recommendations are being questioned in several European countries including Sweden. The recommendation by RAV not to screen for and treat LTBI in persons living with HIV is justified by the fact that Sweden has reached the UNAIDS 90-90-90 targets, and that all patients are required by law to attend regular monitoring of their HIV infection (at least twice per year) (204). The RAV statement is also grounded on the reported low capacity of TST and IGRA screening tests to define a population at high risk of developing TB. The suggested strong protective effect of early ART (and low predictive value of screening) gains support from a European multi-center study from 2014 which shows that none of the study's 338 patients with HIV with viral suppression at the time of LTBI screening developed TB during the following years (211). The fear that combining ART and LTBI treatment (INH) would lead to more AEs could not be confirmed in a Cochrane meta-analysis from 2015 (212).

In our cohort we estimated that 22 people living with HIV in Sweden who had their origin from a TB-endemic country, had to be treated with chemoprophylaxis to prevent one case of late active TB. This figure is significantly higher than the estimated NNT in the formerly cited Swiss study, where only 8 patients (during a median follow up time of 4.4 years) with HIV, with an origin from endemic countries and a positive LTBI screening test had to be treated to prevent on active case of TB (210). However, our Swedish cohort results align with the estimated NNT in the recent systematic Cochrane review of TB endemic countries where the overall NNT for LTBI-treatment in TST positive persons living with HIV was estimated to 20 patients (213). Important to note is however that the estimation of NNT is very much dependent on length of follow-up. While most patients in the studies from endemic countries were followed for only a year or two, the patients in our study were followed for a median time of 7.4 years. The importance of follow up time for the comparison of NNT is highlighted by the recent British study that report a seemingly high NNT of 264 to prevent one case of TB annually. Had we though instead reported our results as annual NNT and as the British study assumed a restricted protective isoniazid effect of 60%, we would end up with roughly the same estimate that 271 high risk patients would need to be treated with chemoprophylaxis to prevent one case of active TB per year.

In our study the majority of TB patients with active TB were diagnosed at or within 1 month from HIV diagnosis. The prevalence of active TB 1 month after HIV diagnosis (2.6%) in our cohort was significantly higher than found in a British study covering the time span 2011–2015 (0.9%). This latter observation is in congruence with a recent Swedish study which shows that an increasing proportion of persons living with HIV are diagnosed late and present with advanced disease. It should be emphasized that preventing TB, even in the era of ART, must include generous HIV screening and testing routines in the community.

5.4 PAPER IV

5.4.1 Results

A total of 3,344 TB patients were registered in Stockholm County 1996–2016. Culture verification was obtained in 2,552 cases. Among the 2,552 culture-positive cases, 54% were male, 82% were of foreign origin, and 53% were originally from an area with a TB incidence of $\geq 100/100,000$ population. The median age at TB diagnosis was 35 years (IQR 26–51).

The annual numbers of culture-verified cases ranged between a minimum of 85 in 2004 to a maximum of 184 in 2009 (see Figure 1 in Paper IV). The median follow-up time in the study was 10.2 years (IQR 5.6–16.2). TB reoccurred more than 180 days after successful treatment completion in 60 patients. The second episode was culture-confirmed in 24/3,344 cases (0.7%). Out of the 24 cases with a culture-confirmed second episode the isolate could be obtained, and WGS analysis revealed that 12 cases were caused by relapses and five were caused by reinfections. **Figure 8.**

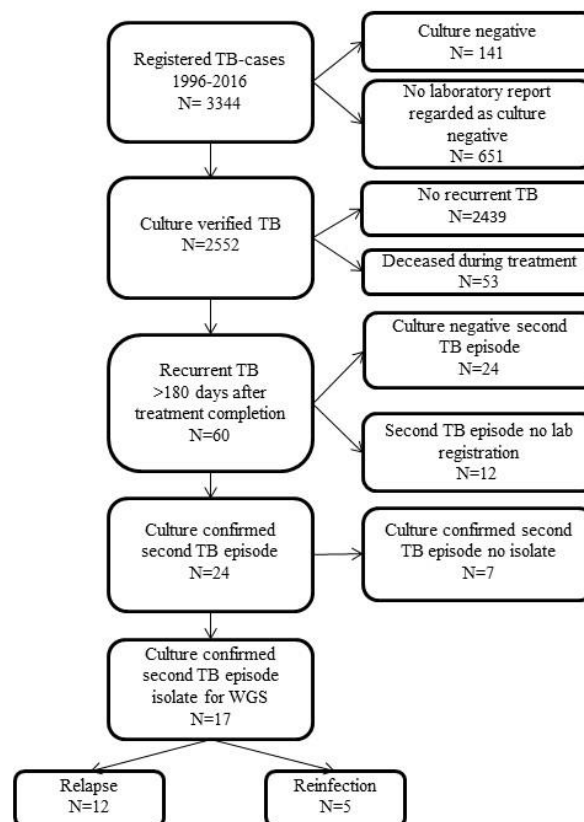


Figure 8. Flow chart of culture-verified tuberculosis cases in Stockholm 1996–2016.

In the group of 24 culture-verified recurrent cases the median age was 24 (IQR 19–38) years; 12 (50%) were women, 20 (83%) were born abroad and 15 (62%) were originally from a region classified as a TB-endemic ($\geq 100/100,000$ population). The median age was significantly lower ($p=0.04$) in the group of patients with TB recurrence (see Table 1 in Paper IV). In a bivariate logistic regression analysis we found no significant associations between TB recurrence and clinical characteristics at TB diagnosis. **Table 8.**

Table 8. Results from logistic regression analysis with TB recurrence as dependent variable by characteristics in persons diagnosed with TB in Stockholm County 1996-2016 (n=2,552).

	Non TB recurrence (n= 2528)	TB recurrence (n=24)	Bivariate analysis OR (95% CI)	p-value	Multivariate analysis OR (95% CI)	p-value
Age (years)						
<35	1323	17	Reference			
≥35	1205	7	2.21 (0.91–5.4)	0.08*	2.20 (0.91–5.34)	0.08
Sex						
Female	1162	12	Reference			
Male	1366	12	1.18 (0.53–2.63)	0.52	1.16 (0.52–2.59)	0.72
TB-incidence in country of origin						
<100/100,000	1196	9	Reference		Not included	
≥100/100,000	1332	15	1.57 (0.46–5.38)	0.47		
Clinical manifestation of TB						
Disseminated	308	5	Reference		Not included	
Pulmonary	1335	12	2.46 (0.72–8.47)	0.15		
Extrapulmonary	885	7	2.30 (0.61–8.61)	0.22		

OR, odds ratio; CI, confidence interval. Pulmonary; only lungs involved, Extrapulmonary; lungs not involved, Disseminated; both pulmonary and extrapulmonary involvement. *Variables with a *p*-value >0.10 in bivariate analysis were not included in the multivariate analysis except for sex, which was considered to be a potential confounding factor.

In the recurrent cases (n=24) the median time between the first and the second TB episode was 3.5 years (IQR 1.8–5.8). No case of TB recurrence was noted in patients diagnosed with their first TB episode 2011–2016. **Figure 9.**

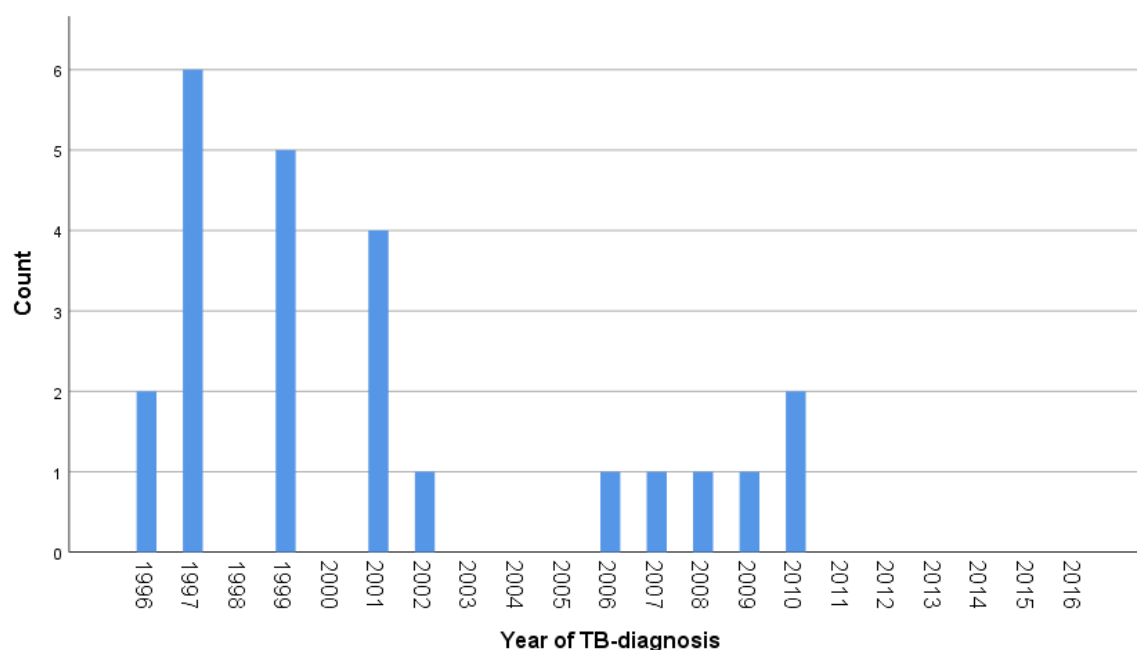


Figure 9. Year of diagnosis, first TB episode, in patients with later recurrent TB (n=24).

In patients analyzed with WGS (n=17) the median time between treatment completion and the second TB episode was 2.4 years (IQR 1.1–4.7), in relapse cases (n=12) and in reinfected cases (n=5) 5.4 years (IQR 3.4–11.3). **Figure 10.**

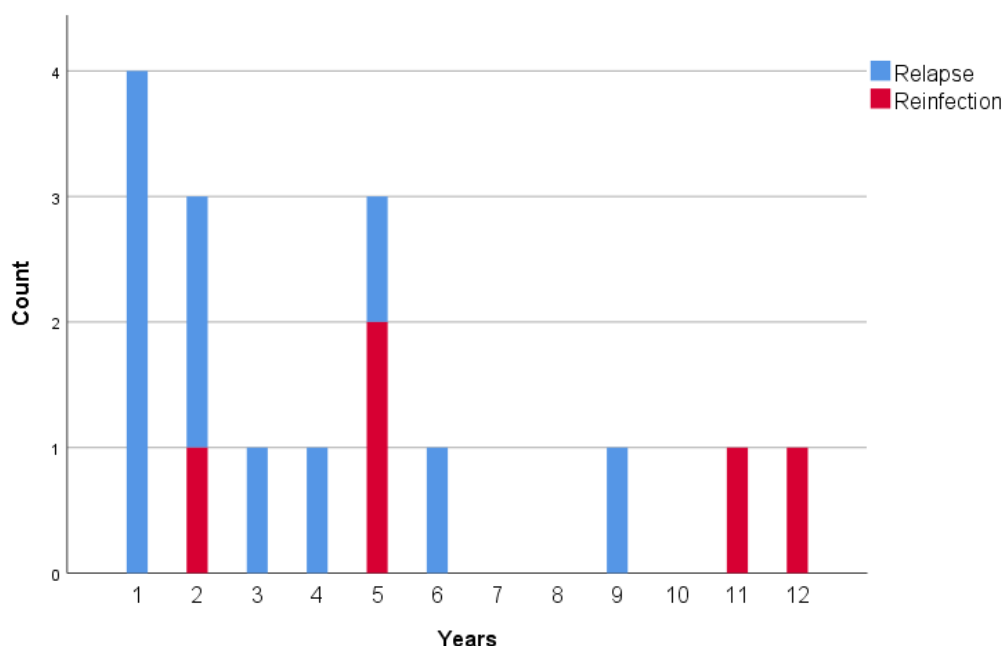


Figure 10. Time from completion of treatment for the first TB episode to confirmation of the second TB episode in patients analyzed with Whole Genome Sequencing, separated in relapse and reinfection (n=17).

5.4.1.1 Results of WGS

The *M.tb* isolates from the 17 patients with recurrent TB underwent WGS. The Euro-American lineage was the most common, seen in 71% of all recurrent cases and 75% in all relapse cases. The number of SNPs differed from none to 1439 between the first and the second isolate. Although a relapse case was defined as having a maximum difference of five SNPs between the two isolates, for relapse case 1 (**Table 9**) a difference of seven SNPs was observed, but none of these seven SNPs were located in highly variable regions. For relapse case 3, the initial SNP analysis revealed a 125 SNP difference. However, further analysis showed a low frequency contamination with a non-mycobacterial species. When the SNP ratio cut-off was increased to 96%, the contamination was removed and only one SNP remained. Thus, 12 cases (71%) were classified as relapse and five were regarded as reinfection with a new strain, resulting in a relapse frequency of 0.5% for the whole study period, corresponding to an annual risk of 0.06% per year. **Table 9.** Drug resistance was present in nine (53%) of the cases with recurrent TB. In the 12 patients with TB relapse, six (50%) patients had a susceptible strain, four (33%) had an INH resistant strain and two (17%) had an MDR strain. No acquired drug resistance was detected in isolates of the second episode among relapse cases. **Table 9.**

5.4.1.2 Risk factors for TB recurrence

Among patients with relapse, four (36%) had adherence problems, five (46%) were smokers and two (18%) had alcohol or drug abuse. One patient with several risk factors for TB relapse, relapsed (3 SNPs difference) after 10 years. In the group of reinfected patients, all were foreign born and two (33%) had traveled to their country of origin the year before the second episode. None of the patients with TB recurrence was co-infected with HIV.

Table 9. Lineage and number of Single Nucleotide Polymorphisms between isolates from first and second TB episode.

Case number	Country of origin	Number of Single Nucleotide Poly-morphisms	First episode Drug resistance gene mutation	Whole Genome Sequencing Lineage first episode	Second Episode Drug resistance gene mutation	Whole Genome Sequencing Lineage second episode
Relapse 1	Sweden	7	<i>katG</i> : nt 1-1071 deleted	lineage4.1.2.1 Euro-American	<i>katG</i> : nt 1-1071 deleted	lineage4.1.2.1 Euro-American
Relapse 2	Sweden	0		lineage4.1.2 Euro-American		lineage4.1.2 Euro-American
Relapse 3	Sweden	1 (125) ^a		lineage4.3.3 Euro-American		lineage4.3.3 Euro-American
Relapse 4	Afghanistan	0		lineage3.1 East-African-Indian		lineage3.1 East-African-Indian
Relapse 5	Sweden	3		lineage4.3.3 Euro-American		lineage4.3.3 Euro-
Reinfection 1	Peru	311	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu	lineage4.3.3 Euro-American		lineage4.3.3 Euro-American
Reinfection 2	Uzbekistan	155		lineage2.2.1 East-Asian		lineage2.2.1 East-Asian
Reinfection 3	Kenya	1317		lineage3.1 East-African-Indian	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American
Reinfection 4	Peru	303	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu	lineage4.3.3 Euro-American		lineage4.3.3 Euro-American
Relapse 6	Somalia	1	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American
Relapse 7	Azerbaijan	0	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu <i>embB</i> : Gly406Asp <i>pncA</i> : 286-InsT	lineage2.2.1 East-Asian	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu <i>embB</i> : Gly406Asp <i>pncA</i> : 286-InsT	lineage2.2.1 East-Asian
Relapse 8	Somalia	4	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American
Relapse 9	Somalia	1	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American	<i>inhA</i> : C-15T	lineage4.6.1.2 Euro-American
Relapse 10	Somalia	3	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu <i>embB</i> : Met306Val	lineage4 Euro-American	<i>katG</i> : Ser315Thr <i>rpoB</i> : Ser450Leu <i>embB</i> : Met306Val	lineage4 Euro-American
Relapse 11	Somalia	1		lineage2.2.1 East-Asian		lineage2.2.1 East-Asian
Reinfection 5	Mongolia	1439	<i>inhA</i> : C-15T	lineage4.3.3 Euro-American		lineage2.2.1 East-Asian
Relapse 12	Bolivia	3		lineage4 Euro-American		lineage4 Euro-American

^a This sample was contaminated with another bacterial species. If the SNP ratio cut-off was increased to 96%, only one SNP remained.

5.4.2 Discussion

We showed that only 0.7% of the TB patients diagnosed in Stockholm County between 1996 and 2016 had a recurrent, culture-confirmed infection during a median follow-up time of more than ten years. There was an indication of a somewhat higher proportion of reinfection in Stockholm than presented in a contemporary Finnish study. One explanation for this could be that the majority of TB patients in Stockholm (82%) originated from high-endemic regions, whereas only 14% of the patients in the Finnish cohort were of foreign origin. A higher prevalence of TB in some immigrant communities, in combination with socioeconomic risk factors such as crowded living conditions and long stays in refugee camps could be expected to increase the risk of reinfection. More frequent travel to TB-endemic regions among immigrants is probably associated with a higher risk of reinfection. Careful contact tracing is of utmost importance to limit the risk of new TB cases.

M.tb has been reported to have a high genomic stability with a steady mutation rate of around 0.3–0.5 SNPs per genome per year. With a five-SNP cut-off for the definition of relapse, the WGS method has been shown to better discriminate relapse from reinfection than RFLP, MIRU-VNTR and spoligotyping. Some cases earlier regarded as relapse, are classified as reinfection with WGS (157). Also, WGS has better capacity to identify minority *M.tb* strains within in populations, which may explain the previous difficulties in distinguishing relapse from reinfection in patients with a mixed primary infection (165).

We observed an almost 4-fold increased frequency of resistant TB strains among patients with relapse and reinfection compared with the mean frequency of TB resistance reported in Stockholm County during the later part of our study period, although we could not accurately estimate the strength of the association. Our observation is in consistency with former studies from low endemic settings showing resistant *M.tb* seems to be a strong risk factors for TB recurrence (214). With the addition of drugs and prolonged treatment, according to the WHO recommendations, the outcome improves. In Sweden, patients with MDR TB are followed for two years after treatment completion to identify relapse at an early stage. Implementation of a prolonged follow-up time might be worth considering also for patients with mono drug resistant TB. The high frequency of resistant TB in patients with reinfection is harder to explain. Traveling back to regions with a high burden of resistance could be one reason.

No case of TB recurrence was observed during the last six years of the study period. As we could not accurately calculate the patient time at risk, we do not know if this is a true reduction, but it might indicate improved TB care during recent years.

6 ETHICAL CONSIDERATIONS

Not harming the study subjects and not restricting their autonomy are our most important ethical considerations when conducting our studies.

Studies I, III and IV were of retrospective character and the study objects were not directly involved. We did not retrieve the subjects' approval in these studies as it would likely have resulted in a decrease in inclusion, especially since many subjects had returned to their country of origin and some had died. Clinical data were retrieved from medical files and registers, and neither treatment nor medical care of the subjects involved could be affected in any way. All data were anonymized and coded before analysis and only persons directly handling the patients' data had access to the code. The studies were approved by the Regional Ethical Review Board of Stockholm (Dnr: 2006/1445-31/, 2013/2184-32, 2015/1300-32 and 2018/1171-31/1).

Study II was a phase 1 trial where the study subjects received two injections with active vaccine or placebo. The vaccine had earlier been proved safe when administered to animals. The study subjects were selected according to the inclusion criteria, and were all found to be healthy at medical examination. They all gave written informed consent at inclusion in the study. The subjects were followed for AEs for 182 days. Blood samples were stored according to the Swedish national rules and guidelines for human biological samples (the biobank law 2002:297). All data regarding the subjects were anonymized and coded. Only persons conducting the study had access the code. Data were registered in a Case Report Form and in patients' files. The study was approved by the Regional Ethical Review Board of Stockholm (Dnr: 2007/716-31/4, 2007/1341-32). Several subjects experienced a skin reaction at the site of the earlier TST on the forearm. The TST was then withdrawn from the inclusion procedure after an amendment, approved by the Regional Ethical Review Board (Dnr: 2008/92-32).

7 CONCLUSIONS

- The introduction of ART increased the success of treatment for active TB in patients with concomitant HIV infection. However, patients that were provided combined ART and TB treatment were more often burdened by adverse drug reactions than patients who had not yet started ART. The highest risk was observed in patients who initiated ART during ongoing anti-TB treatment.
- Our study showed that the TB vaccine candidate H4:IC31 has an acceptable safety profile and is immunogenic in previously BCG-vaccinated healthy individuals. Two vaccinations with a low dose of H4 antigen (5-50 µg) in combination with a high dose of the IC31 adjuvant (500 nmol) induced the strongest T-cells response.
- The incidence of active TB in persons living with HIV in Stockholm County declined significantly after the introduction of ART in 1996 but was still 80 times higher than in the general population at the end of the study period, 2016.
- The frequency of relapse in patients who had completed the prescribed TB treatment regimen in Stockholm County was low, indicating well functioning TB care. WGS seems to be a useful tool to distinguish relapse from reinfection and could be used to improve care of TB patients and disease control measures. The high proportion of resistant TB strains in patients with relapsing infection indicates a need of better treatment control and longer follow-up time in this group.

8 FUTURE PERSPECTIVES

To reduce the burden of tuberculosis worldwide it is of utmost importance to detect and treat patients with active TB early in their disease course. To achieve the WHO End TB Strategy goals, TB treatment must also be offered to persons with LTBI to prevent later development of active infection. However, most persons with LTBI will not develop active infection. At present, this means that many patients are unnecessarily exposed to months of antibiotic treatments associated with potentially severe side effects. Improving methods that can better identify persons at risk of developing active infections should be a priority. A more rapid and accurate diagnostic test for active TB is needed for both high- and low-endemic settings. WGS might in the future be an aid for an on-the-spot test, identifying mutations known to be associated with antibiotic resistance so that the patient can receive the correct treatment quickly.

People living with HIV are vulnerable. Screening and treatment of LTBI in persons with HIV infection could be expected to substantially reduce the risk of active TB in this group, in both high- and low-endemic regions. Treatment regimens for LTBI based on shorter treatments with fewer and/or less severe side effects are under development and will probably make this approach more attractive even in the era of ART. Expanded HIV screening for earlier HIV diagnosis and early implementation of ART is also of utmost importance to further reduce the incidence of TB-HIV co-infection in the future.

The increasing problem of resistant TB demands new tuberculosis vaccines that are safe, affordable and more effective than BCG. The promising results with the M72/AS01 vaccine, indicating significant protection against tuberculosis disease, are encouraging. However, future research will be needed on this and other vaccine candidates before they can contribute to the fight against the global TB epidemic.

The patients' integrity must always have the highest priority. To achieve collaboration with the patients and to overcome linguistic, cultural and socioeconomic barriers is a huge challenge – but it also makes the job worthwhile.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Tuberkulos (tbc) är en smittsam infektionssjukdom som under årtusenden har plågat mänskligheten. Sjukdomen har i alla tider framför allt drabbat fattiga och socialt utsatta personer. Undernäring och annan sjuklighet kan medföra att tbc-förloppet förvärras. Trångboddhet ökar risken för smittspridning. Med förbättrade levnadsförhållanden och med upptäckten av effektiv antibiotikabehandling i mitten av 1900-talet minskade förekomsten av tbc i drastiskt höginkomstländer. Även i Sverige var tbc tidigare en farsot men den är idag en ovanlig sjukdom. Ökad invandring från områden där tbc är vanligare, har dock medfört att sjukdomen blivit något vanligare i Sverige de senaste åren.

Tbc orsakas av bakterien *Mycobacterium tuberculosis* (*M.tb*). Spridningen från den sjuka individen sker med hostningar och nysningar, så kallad luftburen smitta. När dropparna inandas av en annan människa och når lungorna försöker immunförsvaret döda bakterierna. Bakterien har utvecklat ett försvar mot dessa attacker och lyckas ofta överleva i kroppen i en inaktiv form, så kallad latent tbc. Personer med latent tbc är varken sjuka eller smittsamma. Senare i livet, oftast inom några år efter smittotillfället, kan bakterierna "vakna" och orsaka aktiv och smittsam sjukdom. Detta drabbar ungefär 10 % av de i övrigt friska personer som smittats med tbc-bakterien.

M.tb har flera egenskaper som gör att den är svår att avdöda. Vid aktiv tbc behandlas patienten med ett flertal antibiotika under minst sex månader. Personer som är bärare av latent tbc behandlas oftast med ett eller två läkemedel under tre till nio månader. Tbc-läkemedel kan orsaka biverkningar. Leverpåverkan är den mest allvarliga biverkan men behandlade personer kan även drabbas av svåra besvär från, nervsystemet, mag-tarm-kanalen, leder och hud. Dessa faktorer gör det ibland svårt för patienten att följa behandlings-ordinationerna.

Vid infektion med humant immunbristvirus (hiv) försvagas den del av immunförsvaret (CD4⁺ celler) som är viktigast i skyddet mot tbc. Hiv smittade personer löper därför en ökad risk för att insjukna i aktiv tbc. Hiv började spridas över världen under 1980-talet vilket ledde till att även förekomsten av och dödligheten i tbc ökade under efterföljande decennier. Värst drabbat är Afrika söder om Sahara. Antiretroviral behandling (ART), som effektivt hämmar virusets förmåga att föröka sig, infördes i den rika delen av världen på 1990-talet. Hiv är numer en behandlingsbar om än ännu inte botbar sjukdom men ART är fortfarande inte tillgängligt för alla hiv-drabbade personer i världen. Risken för att drabbas av biverkningar av tbc-behandlingen är större för personer med HIV som samtidigt behandlas med ART.

Trots att botande behandling mot tbc finns sedan 1960-talet så är sjukdomen fortfarande en av de vanligaste dödsorsakerna i världen. Världshälsoorganisationen (WHO) rapporterade 2017, 10 miljoner nya fall av tbc och 1,6 miljoner dödsfall. Av de döda var 300 000 även infekterade med hiv. Ökad förekomst av resistent tbc det senaste decenniet har försvårat världssamfundets möjligheter att begränsa sjukdomens spridning. Trots dessa utmaningar formulerade WHO 2014 målsättningen att minska förekomsten av tbc med 90 % år 2035. För att uppnå målet måste tbc-epidemin angripas på flera olika sätt.

- Ett förbättrat vaccin mot tbc skulle vara ett oerhört viktigt redskap i den globala kampen mot sjukdomen. Det sedan länge använda tbc-vaccinet Bacille-Calmette Guérain (BCG) ger visserligen ett gott skydd mot tbc hos barn under fem år men har otillräcklig effekt hos vuxna.
- Kortare behandlingstider med mindre biverkningsframkallande läkemedel skulle leda till färre behandlingsavbrott och därmed förbättrade behandlingsresultat bland patienter med både latent och aktiv tbc vilket också skulle leda till kraftig minskning av spridningen av infektionen.

- De flesta personer som bär på *Mtb* kommer inte att utveckla aktiv sjukdom. Förbättrade diagnostiska metoder som kan avgränsa de personer med latent tbc som löper särskilt stor risk för att utveckla aktiv tbc skulle kunna bespara många individer och samhället verkningslös, biverkningsbehäftad och resistensdrivande antibiotikabehandling.

Studier i låginkomstländer har visat att strukturerade insatser för att upptäcka och behandla personer med latent tbc leder till kraftig minskning av insjuknande i aktiv tbc och då framför allt hos personer med samtidig hiv-infektion. WHO rekommenderar därför screening och behandling av latent tbc hos alla personer som lever med hiv. Den svenska Folkhälsomyndigheten rekommenderar i första hand behandling av latent tbc hos hiv smittade personer som ännu inte har påbörjat ART. Sverige har förhållandevis låg förekomst av hiv men migranter från vissa områden i världen är mer drabbade. Hiv-vården i Sverige är mycket välfungerande. Den svenska Referensgruppen för AntiViral terapi (RAV) gjorde 2017 uppskattningen att mer än 90 % av alla de som levde med infektionen hade effektiv ART-behandling och välfungerande immunförsvar (normala CD4⁺ cell). RAV har därför beslutat om att inte rekommendera behandling av latent tbc då de anser att den förmodligen gör mer skada än nytta.

Syftet med denna avhandling var att öka kunskapen om tbc, med betoning på förebyggande behandling, i ett lågendemiskt område med ökande migration från högendemiska länder.

I den första studien beskrevs förekomsten av tbc och de biverkningar som uppstod under tbc-behandlingen hos personer med som lever med hiv i Stockholms län sedan hiv-epidemiens början. I studien, som spänner över 1987 till 2013 fann vi att förekomsten av tbc var högst bland migranter från endemiskt tbc-område och att förekomsten minskade betydligt efter införandet av ART 1996. Personer som startade behandling med ART under pågående tbc-behandling och personer med dåligt immunförsvar (låga CD4⁺ celler) fick mest biverkningar. De vanligaste biverkningarna var leverpåverkan och neurologiska symptom.

I den andra studien undersökte vi om ett nytt tbc-vaccin var säkert för människor och gav upphov till önskvärd reaktion från immunförsvaret. Tidigare BCG-vaccinerade försökspersonerna lottades till att få aktivt vaccin eller placebo och följdes avseende biverkningar. För att undersöka reaktionsmönstret i immunförsvaret togs blodprover. Vi kunde visa att vaccinet inte orsakade fler allvarliga biverkningar än placebo och att en reaktivitet hos immunförsvaret kvarstod ännu ett halvår efter den sista injektionen med aktivt vaccin. Även om dessa resultat är lovande så måste, för att uttala sig om vaccinet eventuella skyddseffekt, vaccinet prövas på ett stort antal människor i ett tbc-endemiskt område. Personerna måste sedan följas under flera år.

I den tredje studien ville vi titta på behovet av behandling av latent tbc hos personer som lever med hiv i Stockholm sedan införandet av ART 1996 till 2016. Vi fann att tbc var 80 gånger vanligare bland personer med HIV, med ursprung från tbc-endemiskt område, jämfört med normalbefolkningen. Bland de patienter som insjuknade i tbc, hade merparten (77 %) inte effektiv ART. Utifrån studieobservationerna kunde vi beräkna att ungefär 22 patienter, bland de med ursprung från ett tbc-endemiskt område, behöver behandlas för latent tbc för att ett fall av aktiv tbc skall kunna undvikas. Studien besvarar inte frågan om personer med hiv-infektion som bär på tbc i ett lågendemiskt land verkligen bör behandlas med långa antibiotikakurer mot tbc men studien tillhandahåller ett konkret underlag för det fortsatta arbetet med att väga nyttan mot risken med sådan behandling så att vården kan utformas på bästa sätt för både tbc smittade individer och för samhället.

I den fjärde studien ville vi, genom undersökning av arvsmassan hos tbc-bakterien, så kallad helgenomsekvensering (WGS), se om personer som återföll i tbc efter slutförd behandling i Stockholms län 1996-2016, återinsjuknade med samma stam eller om de var nyinfekterade

med en annan stam. Vi kunde visa att endast 0,7 % av alla personer som drabbats av aktiv tbc kom att återfalla i infektionen under de följande 10 åren och att återinsjuknande med samma stam var vanligast. De flesta som återföll var infekterade med en resistent tbc-stam. Resultatet tyder på att tbc-vården fungerar väl men att uppföljningstiden för personer med resistent tbc kan behöva förlängas så att de som ändå återinsjuknar upptäcks i tid.

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